Indian Agricultural Research Institute, New Delhi

COLLECTED PAPERS

of the

Canadian Committee on Food Preservation

containing

Papers 171 - 230

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CANADA

Volume 4 — 1947 - 1949

FOREWORD TO THE FIRST VOLUME

The urgency of overcoming agricultural production problems, such as drought and disease, and the dominance of wheat among Canadian agricultural exports, have tended to relegate research on the preservation, storage, and transport of perishable foodstuffs to a position of secondary interest and attention.   Of late years the volume of wheat exports has diminished and conversely the exports of meats, fish, fruits and vegetables have increased, so that for the years 1937-39 inclusive, the average annual values were, for the wheat, $107,628,000, and for the perishable foodstuffs, $85,282,000.   With increasing difficulty in disposing of wheat surpluses, the trend should be to enlarge our exports of these other products to the maximum possible.   Moreover, the production of most perishables being seasonal, preservation is essential to our domestic as well as to our export trade.   There is thus every reason to encourage and support these institutions and scientists who have embarked upon a programme of investigations in this field.

Part of the lag in research on food preservation has been due to a lack of the experimental cold storage and other rather expensive laboratory equipment required. While this deficiency is gradually being rectified, it is likely to be a limiting factor for some time yet.   It is therefore gratifying to find in the institutions concerned a disposition to pool their resources as far as this may be practicable and necessary to the realization of their common aims.   The volume of Collected Papers is part of the fruits of this co-operation.

The Canadian Committee on Storage and Transport of Food grew out of a conference on cold storage held in Ottawa in June, 1934.   Its general objective is to promote and co-ordinate Canadian investigations designed to increase the storage life and improve the quality of perishable products which must be stored for domestic consumption or transported to markets in other countries.   To deal satisfactorily with the diversified problems coming within its scope, the Committee is organized in four sections, dealing respectively with (1) fruits and vegetables, (2) meats and meat products, (3) fish, and (4) engineering problems.   Small panels for the study of taints and refrigerated railway cars have been set up under the section on engineering.

The Committee is sponsored by the National Research Council of Canada, the Dominion Department of Agriculture, and the Fisheries Research Board of Canada. The first of these organizations is studying in its own laboratories the handling and storage of meats, canning problems, and engineering problems.   The Department of Agriculture, through its Experimental Farms laboratories, is investigating the processing and storage of fruits and vegetables.   The investigation of problems in the preservation and transport of fish is of course a responsibility of the Fisheries Research Board.   The Committee has also the co-operation of the Ontario Agricultural College, where a programme of studies on fruit and vegetable storage, designed to utilize the joint facilities of that institution, the University of Toronto, and the Horticulture Experiment Station at Vineland, is under way.

Beginning with 1937, the Committee has issued mimeographed annual reports, consisting of the summaries of the year's progress contributed by members to the annual meetings.   This was intended to give to workers in the same field, particularly in other parts of the British Commonwealth, advance information on Canadian studies under way or projected, thus to encourage direct contacts and exchange of information between workers.   At the same time the Committee initiated a numbered series of papers, reprints of which can be bound together in volumes to be placed in the libraries of institutions conducting food storage investigations.   The number of copies of each volume to be bound is not great enough to make them available to individuals.   The first volume was issued with the hope that it may prove a convenient and useful reference both to members of our own Committee and to workers in other places.

ROBERT NEWTON, CHAIRMAN,
Canadian Committee on Storage and Transport of Food.

February 5, 1941.

FOREWORD TO THE FOURTH VOLUME

THE conditions giving rise to the formation of the Canadian Committee on the Storage and Transport of Food are given in the foreword to Volume 1, which contains the papers submitted from the time the Committee was formed in 1937 to the time of publication in February, 1941. The foreword to Volume 2, which covers the period from February, 1941 to November, 1944, mentioned the expansion of the Committee's activities under the influence of war, and its change in name to the Canadian Committee on Food Preservation. The diversity of the activities undertaken during the war-time emergency was reflected by the papers submitted for Volume 3, which covered the period from November, 1944 to March, 1947. The foreword to this third volume also indicated changes in organization, notably the formation of five subcommittees. While the sponsors remained the same, the number of representatives from industry and the universities was considerably increased.

Since the third volume appeared, the Committee has continued to expand its work. This fourth volume, covering the period from March, 1947 to July, 1949, also contains papers dealing with problems that originated during the war years. During the post-war period, less emphasis has been placed on dehydration as an important means of food preservation (except for certain specialties) and more emphasis has been placed on procedures that maintain the quality desired in a peace-time economy.

W. H. COOK, *Chairman*,
Canadian Committee on Food
Preservation.

September 1, 1949.

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DRIED MILK POWDER

VII. THE EFFECT OF SEASON OF PRODUCTION ON KEEPING QUALITY[1]

By Jesse A. Pearce[2] and W. A. Bryce[2]

Abstract

Milk powders of 1% butterfat content, produced in the fall of the year, had higher initial palatability scores than similar powders prepared in the spring. The skim milk powders from fall milk decreased in quality throughout a storage period of 16 wk. at temperatures of 80°, 100°, or 120° F. Similar powders from spring milk stored at 80° F. increased in quality throughout the storage period while those stored at 100° and 120° F. first increased and then decreased in quality. Powders of 26 or 28% butterfat, produced in the spring or in the fall, had equal initial palatability scores and when stored deteriorated equally. Fall milk powder containing 30% butterfat was better initially than the comparable spring sample, but, when stored, quality changes in both types were about equal. At each storage temperature all whole milk powders deteriorated at about the same rate.

Introduction

In conjunction with investigations of some of the factors affecting deterioration of milk powder, e.g., cooling subsequent to drying (5), exposure to light (6), storage temperature (1), method of packing (7, 11), moisture content (1), and producer (1), it was deemed advisable to evaluate the difference in the behaviour of milk powder produced at different seasons of the year.

It has been shown that liquid milk obtained during the fall and winter months has a higher solids content than spring and summer milk (2, pp. 29-31; 4, p. 405; 10, pp. 55-61), that spring milk is poorest in fat and fall milk the richest (2, 10) and that the fat of milk produced in the fall of the year, when good pasture is no longer available, has a lower iodine value than the fat of spring milk (3). It would be expected, therefore, that fall milk, when dried, would be more stable than powder produced in the spring. This paper describes the results of a storage study on samples of spring and fall milk powders of different fat levels but of equal moisture contents.

Materials and Methods

The spring and fall milk powders used in this experiment were spray-dried and were produced during the latter part of May and early in December, 1944. The butterfat levels compared were 1, 26, 28, and 30%. The moisture content of the powders was adjusted to 2% before packing in tin-plate containers with air as the headspace gas. The palatability of the powders was determined initially and after 2, 4, 8, and 16 wk. storage periods at temperatures of 80°,

100°, and 120° F. (27°, 38°, and 49° C.).   In assessing the quality of the powders, samples were reconstituted as previously described (5) and palatability was assessed by 14 tasters.   Scoring was done on a scale of 10 (the equivalent of excellent fresh whole or skim milk) to 0 (a repulsive specimen).   A score of 4 is usually considered the point at which milk powder is no longer suitable for use as a milk drink.   The reliability of the scoring by the taste panel has been estimated and palatability assessment was found to be more suitable than any of the chemical tests of milk powder quality (5).

Results

The data for the 4, 8, and 16 wk. samplings were assessed by an analysis of variance with the results shown in Table I.   This table shows that no constant difference could be attributed to the use of either spring or fall milk in powder production.   The difference between samples was attributed to the inclusion

TABLE I

ANALYSIS OF VARIANCE OF PALATABILITY DATA ON STORED POWDERS
PREPARED FROM SPRING AND FALL MILK

Variance attributable to:	Degrees of Freedom	Mean square
Seasons	1	1.15
Samples	3	1 12*
Temperature	2	15 30**
Storage time	2	11 93**
Seasons × samples	3	2 45**
Seasons × temperature	2	1.45*
Seasons × time	2	3 83**
Samples × temperature	6	0 25
Samples × time	6	0.83*
Temperature × time	4	1 28*
Residual	40	0.35

   * Exceeds the 5% level of statistical significance.
   ** Exceeds the 1% level of statistical significance.

of data for skim milk powder in the comparison.   Skim milk powders are generally considered to be of poorer quality, when reconstituted as a milk drink, than whole milk powders.   The significant effects of storage temperature and storage time have been discussed in earlier papers, and the other factors of significance can be explained by referring to Fig. 1.

As shown in Fig. 1, skim milk powder (1% fat) from fall milk had a higher initial palatability than skim milk powder from spring milk but decreased in palatability during storage at all temperatures, while the palatability score of skim milk powders from spring milk first increased, then decreased during storage at 100° and 120° F., but at 80° F. increased throughout the storage period.   It is of interest to note that in previous studies, all skim milk samples exhibiting low initial palatability and increasing palatability during storage

have been produced in the spring of the year (5, 6, 7) while the only other general decrease similar to that observed in this study was in a powder produced from fall milk (6). The possibility that this difference in behaviour was attributable to plant practice seems unlikely since powders produced in the spring by two different companies exhibited parallel behaviour (1).
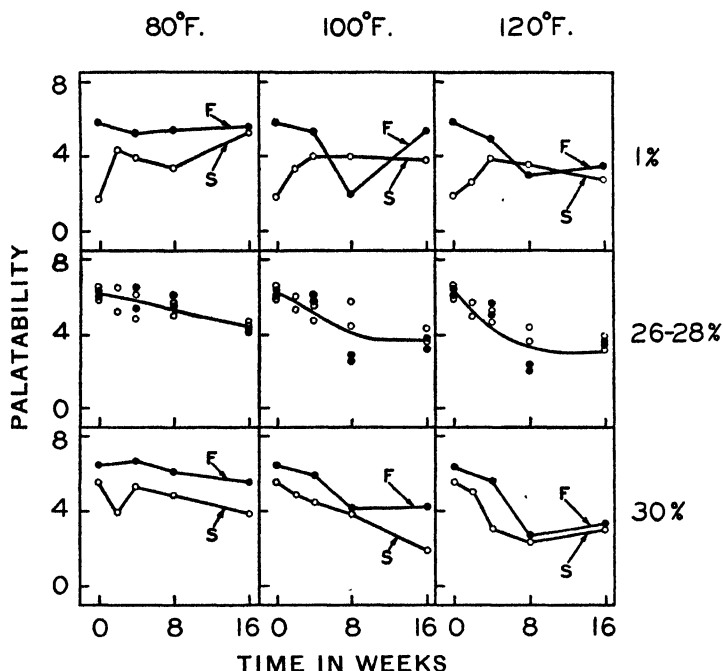
FIG. 1. *The decrease in palatability of stored milk powders prepared from spring and fall milk.*

Whole milk powders having 26 or 28% fat, and prepared from milk produced in the spring or in the fall, did not differ from each other in keeping quality. However, fall milk powder with 30% butterfat had a higher initial palatability than the comparable spring sample. The high-fat, spring powder had a lower palatability than any of the other whole milk powders. This difference may be attributable to some variation in plant practice. Deterioration in both 30% butterfat powders occurred at about the same rate. Deterioration in all whole milk powders showed comparable trends at the same storage temperatures. Changes in whole milk powders were different from those in the skim milk powder from spring milk, but were similar to, although slightly more rapid than, the changes in skim milk powder from fall milk.

Discussion

Experimental work on milk in Finland (10) has shown an increase in fat and protein of milk produced during the summer months and an increase in lactose of that produced during the winter months. Since the change in

composition of milk as cows go to pasture, and vice versa, is not abrupt, it might be expected that the lactose–protein ratio in May would be higher than the ratio in December milk. Calculations on American data (6) show that, for Jersey cows, the ratio of lactose to protein in May is 1.42 compared with about 1.15 for November and December. This makes a difference of 2 5 to 3% in the lactose content of spring and fall dried whole milk and about 4% difference in the lactose content of spring and fall skim milk powders. This, when considered in relation to the high drying temperatures used in preparing skim powders, makes possible some explanation of the difference in behaviour of spring and fall skim milk.

It has been shown that the addition of lactose to a partially defatted milk prior to drying provided some protection to the product during storage (8). However, it is known that lactose deteriorates rapidly when subjected to heat. Spring milk powder might, therefore, have more volatile breakdown products from lactose decomposition during drying than fall milk powder. As suggested previously (6), these degradation products may be dissipated as a result of the repacking operation or of chemical recombination during storage to form substances that do not possess disagreeable flavours. As storage proceeds, the undesirable degradation products would diminish and the preservative effect of the higher lactose content of the spring skim milk powder would become noticeable.

Milk fat from cattle on pasture differs in degree of unsaturation from the fat of milk from partially stall-fed animals (4). If the milks used here conformed in unsaturation to English milks, it would be expected that the butterfat in the fall milk would have an iodine value about six lower than the butterfat in the spring milk. Fall milk powder, then, should be more stable than spring milk powder. Some reflection of this increased stability may have been shown by the higher palatability of the fall milk powder containing 30% fat. However, the deterioration noted in powders of 26 and 28% fat contradicted this and indicated that the butterfat at both seasons was equally susceptible to deterioration.

The facts that packing in inert gases affords only partial protection to whole milk powder (7), that light exerts a harmful effect on skim milk powder (7), and that almost equal deterioration occurred in some whole and skim milk powders point to solids-not-fat as an important factor in milk powder deterioration. The observation that milk powders containing 50 to 55% fat keep 15 to 18 months while powders with 5 to 6% keep only about four months (9) supports this assumption.

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PRESERVATION OF EGGS

V. METHODS FOR DETERMINING YOLK INDEX

By N. E. Gibbons

PRESERVATION OF EGGS

V. METHODS FOR DETERMINING YOLK INDEX[1]

By N. E. Gibbons[2]

Abstract

Breaking out the egg on a glass plate and measuring the height and width of the yolk in position in the white is a rapid and easy method of determining yolk index. The correlation coefficient between this method and one of the more laborious methods, in which the white is removed, is .97. The prediction equation is $y = -0.001 + 0.9172x$.

Introduction

The use of the yolk index as a measure of egg quality was first proposed by Sharp and Powell (3). In their method, the yolk was completely freed of all the white. To remove the last traces of adhering albumen, the yolk was held in the palm of the hand and wiped gently with a cloth. It was then placed on a glass plate, the diameter and height measured, and the yolk index calculated by dividing the latter value by the former. After being placed on the glass plate the yolk continued to flatten for a considerable length of time, but this flattening was most rapid during the first 60 min. However, it was possible to obtain reasonably accurate information if the measurements were made after the yolk had been standing for five minutes. When determining the yolk index by this method, the chances of breaking the yolk are obviously high, especially when storage eggs are used.

The method was modified by Smith (4, pp. 60-61) who left the adherent film of white. This involved less handling and less risk of damage. It was also suggested that the value obtained with the white adhering was of more interest as a basis for judgment of quality. A standard period of two minutes was allowed to elapse before measurements were made.

Further modifications were made at the Low Temperature Research Station at Cambridge (1). As, in this study, comparisons were made with this method it is given in detail: the shell is cracked round by means of a knife or scalpel and the contents carefully transferred to an egg separator. As soon as the major portion of the thick fraction of the white has passed through the opening of the separator, the yolk is allowed to slide into a glass dish containing a sugar solution isotonic with the yolk, i.e., 10.4 gm. of sucrose per 100 gm. of water. While in this sucrose solution the remainder of the thick white and chalazae are removed. The yolk is then placed on a level glass plate, covered with a small beaker or crystallizing dish and allowed to

settle for three minutes.   The height of the yolk is then determined with a
depth micrometer and the width measured across the long and short axes.
The index is expressed as the height divided by the mean width.

In the N.R.C. laboratories it has been the practice to break out the egg
onto a glass plate and measure the height and width of the yolk as it rests
on the white (2).   Practically the yolk is never considered without the white
and as the support given by the albumen lessens with time, as does the strength
of the yolk membrane, it seems reasonable to measure the yolk in this position.
Furthermore the above methods are time-consuming and too often consider-
able time and material are wasted because the yolk breaks at the last moment.
To test the validity of the N.R.C. method a comparison was made between it
and the method used at the Low Temperature Research Station.

The N.R.C. method is as follows.   The egg is broken out onto a clean level
glass plate and the yolk diameter measured immediately with calipers along
the axis bisecting the short and long diameters.   The yolk height is then
measured with a spherometer.   These two measurements are completed
within 30 to 40 sec. of breaking the egg.

Experimental

For comparison, the yolk indices of 182 eggs of various grades and storage
histories were determined first by the N.R.C. method and then by the L.T.R.S.
method.   The scatter diagram and regression line are shown in Fig. 1.   The
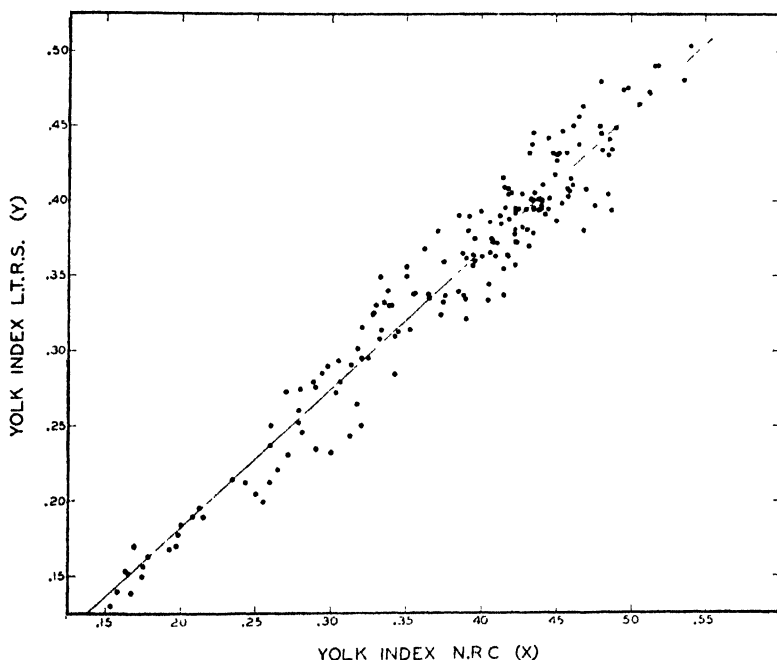
Fig. 1.   *Relation between yolk indices determined by N.R.C. method and the method used
by Low Temperature Research Station.*

correlation coefficient between the two sets of values is .97. The prediction equation is $y = -0.001 + 0.9172x$. The N.R.C. method therefore gives practically the same results as the longer method, and the losses from breakage are negligible.

As may be seen from the regression line the present method gives slightly higher values than the L.T.R.S. method. The white under the yolk contributes slightly to this but the effect decreases as the white thins (Table I). This was shown by breaking the eggs out on a cold brass plate, taking the

TABLE I

EFFECT OF AMOUNT OF WHITE UNDER YOLK ON YOLK INDEX WHEN
MEASURED BY N.R.C. METHOD

	Yolk diameter, in.	Yolk height, in.	Yolk index	Height of white under yolk, in.	Corrected yolk height, in.	Corrected yolk index
Fresh eggs	1.67	.840	.503	.028	.812	.486
	1 68	.780	.464	.017	.763	.454
	1.74	.870	.500	.030	.840	.483
	1 69	.775	.458	.030	.745	.441
	1.70	.730	.429	.031	.699	.411
	1.78	.792	.444	.020	.772	.434
	1.65	.798	.484	.032	.766	.464
	1 69	.877	.519	.038	.839	.496
	1 64	.765	466	.035	.730	.445
	1.62	869	.536	.025	.844	.521
Average	1.69	.810	.480	.029	.781	.464
Commercial Grade *C*	1 63	.695	430	.025	.670	.411
	1 67	.721	432	.040	.681	.407
	1.71	.752	.440	.025	.727	.425
	1.82	.645	.354	.018	.627	.344
	1 61	.686	.426	.007	.679	.422
	1.70	.679	.399	.024	.655	.385
	1.69	.783	.463	.029	.754	.446
	1.82	.815	.448	.040	.775	.426
	1.68	.695	.414	.022	.673	.401
	1.65	.722	.438	.031	.691	.419
Average	1.70	.719	.424	.026	.693	.409
Experimental eggs stored at 70° F.	1.98	.397	.201	.002	.395	.199
	1.82	.585	.321	.011	.574	.315
	1.70	.631	.371	.008	.623	.366
	1.89	.540	.285	.011	.529	.280
	1.76	.602	.342	.001	.601	.341
	1.77	.571	.322	.006	.565	.319
	1.81	.512	.283	.001	.511	.282
	1.78	.605	.340	.006	.599	.336
	1.78	.535	.300	.003	.532	.299
	1.80	.617	.343	.017	.600	.333
Average	1.81	.560	.311	.007	.553	.307

usual measurements, and then placing the plate over a steam jet. The albumen was quickly coagulated, the yolk removed, and the thickness of the albumen measured.

The flattening of the yolk due to the removal of the supporting albumen should also reduce the yolk index. The differences in yolk index of 60 eggs, as determined by the two methods, ranged from 0.066 to 0.020, and this flattening may contribute the greater part of the difference. The effect of both the white under the yolk and the removal of the supporting albumen decreases as the quality of the egg decreases. This is evident from the slope of the regression line.

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PACKAGING

V. THE GREASE RESISTANCE OF SOME COMMON PACKAGING MATERIALS[1]

By C. G. Lavers[2]

Abstract

The grease resistance of a wide variety of packaging materials was tested before and after creasing and ageing, using a modification of TAPPI method T454 m-44. Kraft and sulphite were of little or no value as grease barriers even after paraffin wax coating or impregnating. Vegetable parchment and 'greaseproof' paper were superior to kraft. The grease resistance of glassine was 5 to 10 times greater than that of either greaseproof paper or vegetable parchment. Polyethylene, in turn, was considerably more resistant than glassine, and just slightly less resistant than all grades of "Cellophane", and cellulose acetate, cellulose nitrate, ethyl cellulose, Pliofilm, vinylite, and Saran.

Creasing glassine caused large reductions in its grease resistance, especially when heavy basis weights were tested. Paraffin wax coatings seemed to be more effective in improving grease resistance when dense base stocks were used. On kraft, a heavy wax coating was necessary to produce a small improvement, while on glassine only a very light coating was required, to bring about considerable improvement in grease resistance. Ageing many materials at 140° F. markedly reduced their grease resistance. An exception to this was glassine, most samples of which had greater resistance to grease penetration after ageing. Neither ageing nor creasing appreciably affected the grease resistance of Cellophane, or the thermoplastic films tested.

Introduction

To design a suitable package for a particular food, a knowledge of the mechanical strength, water-vapour resistance, sealing properties, grease resistance, etc., of many packaging materials is usually necessary. Earlier publications in this series have dealt with the water-vapour transmission and mechanical strength of several packaging materials (2, 3). The purpose of the present study is to evaluate the grease resistance of many of the materials commonly used in food packaging.

Materials and Methods

The materials tested included kraft, sulphite, cellucine, glassine, vegetable parchment, manilla, 'greaseproof' paper, "Cellophane", and several thermoplastic films including cellulose acetate, cellulose nitrate, ethyl cellulose, vinylite, polyethylene, Saran, and Pliofilm. Many of the materials were

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examined in both the waxed and unwaxed states and at several different basis weights. A detailed description of all samples studied is given in Table I.

The method of testing grease resistance was essentially that described in TAPPI method T454 *m*-44, with certain modifications. By this method a small pile (5 gm.) of sand is placed on the sample to be tested, to this is added 1.1 ml. of turpentine containing a red dye, and the time required for the coloured turpentine to penetrate the sample and stain a sheet of white paper placed beneath it is measured. The samples were not conditioned and tested at a fixed temperature and humidity as required by T454 *m*-44, all determinations being done at room conditions (approximately 75° F., 35% relative humidity). The TAPPI standard test for grease resistance also states that 30 specimens of each sample should be tested. In this study 15 specimens of unaged, and five of aged, samples were tested. This reduction in the number of specimens tested was believed to be justified because preliminary trials had shown that variations between samples of one type of material from different sources were greater than the variations between different specimens of a single sample. Since samples were available from a single source only and were often taken from the same roll, it was considered that the number of determinations done gave sufficiently accurate values for a particular sample, and for setting up a relative scale for comparing the various materials.

The relative scale used was as follows:

Time for dye to penetrate sample, sec.	Grease resistance score
0 – 50	0
50 – 500	1
500 – 1000	2
1000 – 2000	3
2000 – 4000	4
4000 – 8000	5
8000 – 18000	6
>18000	7

The large number of determinations that had to be done necessitated the adoption of a more convenient procedure than moving each sample to see if penetration had occurred. To overcome this difficulty, a low platform (18 in. high) with a glass top was placed on the laboratory bench. This was illuminated from below with a fluorescent light of suitable length. Samples were then placed on the top of the glass sheet, replacing the white book paper of T454 *m*-44 with a thin tissue that the coloured turpentine penetrated instantly. With this arrangement test specimens could easily be examined for penetration from underneath without moving any samples, making it possible to do many determinations simultaneously.

Since the grease resistance of a creased sheet may be considerably less than that of the uncreased material, samples were tested both before and after

creasing. The method of creasing has been described.* Briefly it consists in making two creases in the material crossing at right-angles by folding first in one direction and then in the opposite, the crease being produced by placing a 1 lb. weight on the loosely folded material.

To simulate long term storage under dry conditions samples were aged for one week at 140° F., low humidity (approximately 6%), conditioned for 24 hr. at room temperature and humidity, and their grease resistance subsequently tested.

Results

Grease resistance scores are given in Table I. Values that are recorded as 7 indicate that penetration did not occur in five hours. Tests were not run longer than this because it was found that at the end of five hours almost all the turpentine had evaporated.

When untreated (i.e., unwaxed, not laminated, and not aged or creased) base stocks were considered, it was obvious that kraft and ordinary sulphite papers were of little or no value as grease barriers. Vegetable parchment and 'greaseproof' paper had approximately equal resistances, and were superior to kraft. Although the values obtained for greaseproof paper were rather low, materials of this type can be produced with very high grease resistance, as shown by the commercial sheets designated in Table I as Lard Liners and Shortening Papers. The very high resistance of these materials was probably a result of the type of pulp used, and the treatment applied to it in the beater (1). The grease resistance of most samples of glassine was 5 to 10 times greater than that of either greaseproof paper or vegetable parchment. Polyethylene was considerably more resistant than most samples of glassine, but less resistant than Cellophane, cellulose acetate, cellulose nitrate, ethyl cellulose, Pliofilm, vinylite, and Saran.

Creasing unwaxed and unaged samples of many materials caused considerable reduction in their grease resistance. This was especially noted with glassine. For this material, the reduction in grease resistance score upon creasing became greater as the basis weight of the sample increased. Creasing did not appreciably affect the grease resistance of Cellophane or the various thermoplastic materials tested.

The effectiveness of paraffin wax in improving grease resistance appeared to depend largely on the nature of the base sheet to which it was applied. On kraft, dry waxing, i.e., wax impregnating, had little beneficial effect. Kraft, paraffin coated on one side, did not show any improvement in grease resistance until a basis weight of 60 lb. per ream (ream weights refer to 500 sheets, 24 by 36 in.) was used with a 12 lb. wax coat. Samples coated on both sides showed some resistance when 25 lb. material was waxed to 35 lb.

* *Wrapping greaseproof. Canadian Packaging Committee Code 105. Sept. 15, 1945. Currently available from Forest Products Laboratories, Department of Mines and Resources, Ottawa, Canada.*

TABLE I

THE GREASE RESISTANCE OF SOME COMMON PACKAGING MATERIALS

Material		Grease resistance score			
		Materials as received (average 15 determinations)		Materials aged one week at 140° F. (average 5 determinations)	
		Flat	Creased	Flat	Creased
Kraft					
50 lb.		0	0	0	0
30 lb. dry waxed to 36 lb.		0	0	0	0
45 lb. dry waxed to 55 lb.		0	0	0	0
15 lb. waxed one side to 20 lb.	Wax up	0	0	0	0
	Wax down	0	0	0	0
28 lb. white, waxed one side to 31 lb.	Wax up	0	0	0	0
	Wax down	0	0	0	0
30 lb. waxed one side to 38 lb.	Wax up	0	0	0	0
	Wax down	0	0	0	0
60 lb. blue, waxed one side to 72 lb.	Wax up	1	0	1	0
	Wax down	3	0	1	0
25 lb. waxed two sides to 35 lb.		2	0	1	0
25 lb. waxed two sides to 50 lb.		2	0	1	0
45 lb. waxed two sides to 65 lb.		3	0	1	0
80 lb. waxed two sides to 105 lb.		4	0	3	0
25 lb. coated one side with a flexible wax composition to 65 lb.	Wax up	5	3	5	4
	Wax down	4	3	5	4
25 lb. wax laminated (5 lb. wax) to 25 lb., thermoplastic coated	Coating up	3	0	0	0
	Coating down	1	0	0	0
Sulphites					
20 lb. dry waxed to 24 lb.		0	0	0	0
30 lb. dry waxed to 38 lb.		0	0	0	0
40 lb. dry waxed to 48 lb.		1	0	0	0
20 lb. unfilled, waxed two sides to 30 lb.		1	0	0	0
20 lb. filled, waxed two sides to 30 lb.		1	0	1	0
Manilla					
48 lb. waxed two sides to 67 lb.		3	0	2	0
Cellucine					
20 lb. waxed two sides to 25 lb.		1	0	1	0
Vegetable parchments					
27 lb.		1	1	1	1
40 lb.		2	1	1	1
27 lb. waxed one side to 34 lb.	Wax up	2	1	2	1
	Wax down	3	1	2	1
27 lb. waxed two sides to 33 lb.		1	1	2	1
'Greaseproof' papers					
20 lb. full-bleached		1	1	1	1
25 lb. full-bleached		1	1	1	1
30 lb. full-bleached		1	1	1	1
35 lb. full-bleached		1	1		
40 lb. full-bleached		3	1	2	1
25 lb. semibleached		1	1	1	1
25 lb. full-bleached, wet strength		1	1	2	1

TABLE I—*Concluded*

THE GREASE RESISTANCE OF SOME COMMON PACKAGING MATERIALS —*Concluded*

Material	Grease resistance score			
	Materials as received (average 15 determinations)		Materials aged one week at 140° F. (average 5 determinations)	
	Flat	Creased	Flat	Creased
Lard liners				
30 lb. full-bleached, semiplastic	3	2	3	1
40 lb. full-bleached	7	7	7	5
Shortening papers				
25 lb. superplastic	4	3	7	7
35 lb. superplastic	5	4	7	7
44 lb. superplastic	7	7	7	7
Glassines				
20 lb. full-bleached	5	3	5	5
25 lb. full-bleached	4	2	3	2
30 lb. full-bleached	6	1	4	3
40 lb. full-bleached	6	1	3	1
25 lb. full-bleached, opaque	2	1	1	1
40 lb. full-bleached, opaque	3	1	1	1
25 lb. full-bleached, opaque, plasticized	2	1	2	1
30 lb. full-bleached, plasticized	5	2	5	4
25 lb. semibleached	3	1	4	3
25 lb. red	3	3	3	3
25 lb. red, plasticized	1	1	3	2
20 lb. amber	3	1	3	3
25 lb. amber	2	1	3	1
30 lb. amber	4	2	4	2
25 lb. yellow	2	2	4	1
28 lb. yellow	3	1	4	1
25 lb. chocolate	1	1	3	2
25 lb. blue	3	3	4	3
25 lb. waxed two sides to 28 lb.	7	7	7	7
55 lb. wax laminated, bleached	7	7	7	7
25 lb. wax laminated to 25 lb. kraft	7	7	7	7
Cellophanes				
300 P.T.	7	7	7	7
300 M.S.T.	7	7	7	7
300 M.S.A.T.	7	7	7	7
300 M.S.Y.T.	7	7	7	7
Thermoplastics				
Polyethylene (0.003 in.)	6	6	6	6
Saran (0.002 in.)	7	7	7	7
Vinylite (0.002 in.)	7	7	7	7
Pliofilm (0.002 in.)	7	7	7	7
Cellulose acetate (0.002 in.)	7	7	7	7
Cellulose nitrate (0.003 in.)	7	7	7	7
Ethyl cellulose (0.005 in.)	7	7	7	7

None of the paraffin waxed kraft samples showed any grease resistance after folding. The kraft sample coated 40 lb. per ream with a flexible wax compound had a much greater transudation time than paraffin coated samples, and retained considerable resistance after folding. The sample of 60 lb. kraft, paraffin coated on one side to 72 lb., showed considerably greater grease resistance when the unwaxed side of the sheet was next to the turpentine; however for the sample coated with a flexible wax compound the opposite was true. The explanation for this is not apparent. Wax laminating two sheets of kraft effected no improvement in grease resistance after folding. Samples of paraffin waxed sulphite were no better than similar samples of waxed kraft.

While only one sample of each of manilla and cellucine were tested, paraffin waxed manilla had a resistance similar to that of waxed kraft, and waxed cellucine was little better than waxed kraft. Paraffin coated vegetable parchment required less wax than kraft paper to effect an improvement in grease resistance but, as with kraft, wax-coated vegetable parchment was no better than the unwaxed material after creasing. The results obtained with samples of paraffin coated vegetable parchment indicated that for a given weight of wax-coating, greater grease resistance could be achieved by putting all the wax on one side of the paper, rather than by dividing it between the two sides. Vegetable parchment, paraffin coated on one side, had greater resistance when the unwaxed side was toward the turpentine. Since paraffin coated kraft behaved in a similar manner it appears that papers coated on one side with paraffin should be used with the unwaxed side toward the greasy surface.

Application of a very light paraffin wax coating to glassine markedly improved its grease resistance both before and after creasing, and wax laminating glassine to glassine, or glassine to kraft, raised the grease resistance score far above that of either sheet alone. The results indicate that the more dense the base stocks, the more effective wax coatings become in enhancing the grease resistance of paper.

Ageing many materials at 140° F. markedly reduced their grease resistance. The major exceptions to this were Shortening papers and glassine, many samples of which had greater resistance to grease penetration after ageing. The resistance of creased samples of these stocks was markedly improved by ageing. When they were aged, much of the wax ran off the kraft samples that were coated with paraffin, and the material assumed the appearance of a dry waxed sheet. Ageing the kraft sample coated with a flexible wax compound did not lower the grease resistance score of the uncreased material, and creased samples had a greater resistance after ageing. The probable reason for this is that the temperature was not high enough to cause the wax to run off the paper, although it was sufficiently high to cause some of it to soak into the sheet. The vegetable parchments, greaseproof papers, Cellophanes, and thermoplastic films tested were not visibly affected by the high temperature storage.

Acknowledgments

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The Nutritive Value of Marine Products
XVI. The Biological Value of Fish Flesh Proteins

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ABSTRACT

The biological values of the crude flesh proteins of lingcod, halibut, lemon sole, and white spring salmon have been determined by growth and nitrogen retention methods using young Wistar rats as experimental animals. Additional values obtained by the growth method are reported for herring and red snapper. For comparative purposes, values were also secured on crude beef flesh proteins, egg albumin, and casein. The figures obtained for the flesh proteins from the four types of fish were of the same order of magnitude, and they were higher than those procured for the other protein foods tested. An examination of the gains in weight was carried out by the co-variance method in which the variance of the actual gains, freed from the effects of varying food consumption by regression, was subjected to analysis. This test indicated that the differences in the average weight gains between the rats fed fish flesh and those fed the other protein foods were highly significant. Data on the effect of sex on the determination of biological values are submitted along with an explanation which helps to resolve conflicting reports on this point.

Recent work demonstrating the deleterious effects of protein deficiency on the healing of wounds (Thompson, Ravdin and Rhoads 1938; Morris, Dubnik and Dunn 1945), resistance to infections (Cannon et al. 1943, 1944), and prevention of chloroform liver injury (Miller, Ross and Whipple 1940) has aroused increased interest in the dietary significance of proteins. Several reviews published in the last year or two deal adequately with these and other aspects of protein nutrition in health and disease (cf. Elman 1944; Stare, Hegsted and McKibbin 1945; and Co Tui 1946). These reports emphasize the desirability of having data readily available on the nutritive value of the main protein food stuffs. A relatively abundant literature exists regarding the proteins of beef, egg, milk, and cereals, but amongst the more important protein foods, least is generally known and published respecting the nutritive properties of fish flesh protiens. Indeed in a number of reference books and reviews on protein nutrition no data whatsoever can be found on these nutrients. In order to supply this information in greater degree the work here reported was conceived and carried out.

At the suggestion of Dr. N. M. Carter, Director of the Pacific Fisheries Experimental Station, four commercially important types of fish were chosen for this study: lingcod (*Ophiodon elongatus*), halibut (*Hippoglossus stenolepis*), lemon

sole (*Parophrys vetulus*), and white spring salmon (*Oncorhynchus tshawytscha*). Additional determinations were subsequently performed on herring (*Clupea pallasii*) and red snapper (*Sebastodes ruberrimus*). Other protein preparations included in the series for comparative purposes were those of beef flesh, egg albumin, and casein. The latter was a vitamin-free product, Labco brand, and the albumin was a pan-dried product from the firm of Canada Egg Products. The beef flesh protein preparation was obtained by processing similar proportions of all the wholesale cuts and was thus roughly representative of the whole steer: round steak 2.5 lb., sirloin 2.5 lb., tenderloin 1.5 lb., stewing meat (flank and shoulder) 6 lb., prime rib roast 2.5 lb. (1 lb. equals 0.45 kg.)

EXPERIMENTAL

The fish were prepared in the form of fillets or steaks and cooked one hour at 99 to 100°C. in a steam autoclave. The cooked product was then pressed, minced, and partially dehydrated in a warm air tunnel at 60°C. The cooking liquors were saved, separated from accompanying oil or fat, and evaporated to a thick syrup in vacuo on a water bath at 40 to 50°C. The concentrate was then re-incorporated into the partially dehydrated minced flesh and the whole re-dried at 50°C. to a moisture content of 2 to 4 per cent. The preparations were then ground into a fine meal. The beef flesh was treated in the same manner except that as much as possible of the fat and discrete connective tissue were discarded before cooking and any observed after cooking was also discarded. This was done since one of the intended features of the investigation was to test only that part of the protein foods which is usually consumed by man. It was assumed that the gross connective tissue did not come under that category. The cooking liquors were retained in the protein preparation because such a procedure simulates best household practice.

In the five feeding tests described below, fresh protein preparations were made for each trial, with the exception of the halibut of trial 5, and the lingcod, lemon sole, and white spring salmon preparations listed in table IV. As a necessary preliminary task, moisture, nitrogen, and fat determinations were carried out on each protein product. The diets (table I) were then made up on the basis of these figures to contain the same amount of crude protein (Nx6.25) and to be of isocaloric value. Nitrogen determinations were carried out on certain of the diets and amounts agreeing closely with the expected values were obtained. The rats were of the Wistar strain and in every case they were divided amongst the different groups according to weight, sex, and litter. They were housed in a room at a controlled temperature of 20 to 22°C. in individual cages having $\frac{1}{4}$-inch mesh screen floors.

The ideal diet utilized to determine the biological value of proteins should be adequate for maximal growth except with respect to protein. Perhaps the most difficult problem confronting anyone attempting to determine the biological value of proteins is the provision of B-vitamins without the introduction of protein material other than that being tested. This obstacle has diminished to some extent in recent years, owing to the preparation of certain of the B-vitamins

in pure form. Evidence is herewith presented to show that under the conditions set forth in this paper, protein is just as efficiently utilized when the following five crystalline vitamins are supplied: thiamine, riboflavin, pyridoxine, calcium pantothenate, and nicotinamide, as when yeast and liver concentrates are supplied as sources of the B-vitamins. In order to demonstrate the adequacy of the basal diet, control diets were fed containing adequate amounts of protein for maximal growth. In three of the feeding trials herein reported, the test protein was fed at a level of 8%, and at 10 and 12% in the remaining trials. The level of 8% was chosen so as to allow a moderate degree of growth which was definitely below the maximum. These conditions have been found to be well suited to the type of work under investigation (Osborne, Mendel and Ferry 1919). A level of 12% test protein was utilized in the fourth feeding trial to determine whether or not the differences in biological values observed at a level of 8% protein were maintained.

Table I shows the composition of the basal (A) and control diets utilized in the feeding experiments.

TABLE I.   Percentage composition of diets

Components	A	B	C	D	E	F	G	H
Test protein	8	8	8	12				
Casein (a)					20	20	20	20
Beef dripping	20	20	10	10	20	20	10	10
Corn oil (b)			10	10			10	10
Sucrose	63	62.9	62.3	58.3	51	45	50.3	48.8
Salts (c)	5	5	5	5	5	5	5	5
Agar	2	2	2	2	2	2	2	2
Cod liver oil (d)	1	1	1	1	1	1	1	1
Vitamin mixture (e)	1	1	1	1	1	1	1	1
Yeast						5		
Yeast concentrate (f)			0.25	0.25			0.25	1
Liver concentrate (g)			0.25	0.25		1.0	0.25	1
Choline chloride	*	0.1	0.20	0.20	0.1	0.1	0.20	0.2

(a) Labco brand, vitamin-free. (b) Mazola. (c) (Beveridge and Lucas 1945). (d) Mead's, 1800 I. U. of vitamin A per g. and 175 I.U. of vitamin D per g. (e) The vitamin mixture is made up of powdered sugar and crystalline vitamins (obtained from Merck) so that 10 g. food supplied 25γ thiamine hydrochloride, 40γ riboflavin, 20γ pyridoxine hydrochloride, 100γ calcium pantothenate, 100γ nicotinamide. (f) Harris Laboratories, Tuckahoe, New York. (g) Eli Lilly and Co., Indianapolis, U.S.A. *Choline chloride included in vitamin mixture so that 10 g. food supplied 10 mg. choline chloride.

FEEDING TRIAL 1

The fact that biological values derived from rats with widely different food intakes possess only doubtful comparative significance is one which is well known (Osborne, Mendel and Ferry 1919). An attempt was therefore made to perform

the experiment on a paired feeding basis. Since some preliminary work had indicated that the lemon sole and lingcod diets were perhaps slightly less palatable than the other rations, the animals were paired-fed on their mates eating the lingcod preparation.

TABLE II. Biological values of proteins of fish and beef flesh and of egg albumin. Average initial wt. of rats: 67.6 g. Range: 40-97 g. Days on test diets: 28. No. of rats per group: 10. Basal diet: A. The rats were paired-fed on the corresponding animals eating the lingcod diet which was given ad libitum.

Source of dietary protein	Daily food intake (g.)	Average gain in wt. (g.)	Gain in wt. per g. protein eaten (g.)
Lingcod	9.6	56.3	2.62
Halibut	9.0	50.3	2.47
Lemon sole	9.0	50.1	2.50
Salmon	9.1	52.0	2.56
Beef	9.0	46.6	2.31
Egg albumin	8.7	45.6	2 34
Control diet E	9.0	76.6	—

In the results (table II), an examination of the food intake reveals that the attempted paired feeding technique was not entirely successful. This was due to the fact that the palatabilities of the diets were essentially the same. The rats on the diet that was fed ad libitum, ate from 5 to 10% more food than did those on the other rations and for that reason greater gains were made and higher biological values observed in this group (cf. Osborne et al. 1919). The gains and biological values induced by the three other fish flesh diets were almost identical and greater than those brought about by the beef flesh and egg albumin diets. It may be noted that the rats on control diet E, identical to the test diets except that it contained an adequate amount of protein for growth in the form of 20% vitamin-free casein, made an average gain of 76.6 grams. This indicated that the chief limiting factor in the test diet was protein.

FEEDING TRIAL 2

The results of the first feeding trial indicated the difficult nature of the task of carrying out successfully the paired feeding technique on animals fed the diets under discussion. Further, it had become obvious that although results such as had been obtained were of some comparative value when arrived at consistently in repeated experiments; nevertheless, the large variations observed in the values within groups made it extremely difficult to demonstrate any clear cut superiority of one protein food over another. The principal factor contributing to the large variation in biological values was found to be the wide range in initial weight. Accordingly rats weighing 50±2.5 grams were used in the subsequent feeding trials. In view of the similarity in palatability of the different diets, it was decided

to let the animals eat ad libitum except when any rat started to consume markedly more than the others. In such cases the amount of food offered was lowered. Only one change, a trivial one to facilitate the preparation of the diets, was made in the basal diet; choline chloride was incorporated in the diet along with the other dry ingredients instead of being included in the vitamin mixture. Since Mitchell and Carman (1926) have shown that the protein content of new tissue is not always the same on different diets, the nitrogen retention method of McCollum and Shukers (cf. McCollum and Simmonds 1929) was utilized. A comparison was thus made possible between the two methods of determining biological values and in addition information was gained regarding the effects of the different diets on the composition of new tissue. Individual nitrogen determinations were performed on a control group of ten rats at the start and on each test rat at the end of the experiment. The intestines were stripped of their contents and the carcasses hydrolyzed with 8 to 10 N H_2SO_4 for 10 to 12 hours. The resulting hydrolysate was made up to volume and aliquots were then pipetted for macro-kjeldahl determinations. A statistical analysis of the percentage nitrogen compositions of the different groups revealed that there were no significant differences. Z value for $p = 0.05$ was 0.8639, found: 0.3724. The average values agreed within a narrow range (2.72 to 2.83%). It therefore appeared that under the conditions herein described, gain in body weight afforded a true indication of protein anabolism. Significant in this respect is the close parallelism of the values obtained by the nitrogen retention and growth methods (table III).

TABLE III.   Biological values of fish and beef flesh proteins. Initial wt. of rats: 50 ± 2.5 g. Days on test diets: 28. Number of rats per group: 10. Basal diet: B (same as basal diet used in feeding trial no. 1 except that choline chloride was not included in the vitamin mixture but added along with the other dry ingredients).

Source of dietary protein	Daily food intake (g.)	Av. gain in wt. (g.)	Gains corr. for food intake (g.)	Gain in wt. per g. protein eaten	% Nitrogen retained of nitrogen ingested.
Lingcod	8.9	61.8	63.0	3.11	56.9
Halibut	9.1	64.4	63.6	3.15	57.7
Lemon sole	9.2	63.0	61.4	3.04	55.4
Salmon	8.9	62.5	63.1	3.12	55.0
Beef	8.9	56.3	56.9	2.80	50.6

A statistical analysis of the gains was carried out by the covariance method in which the variance of the actual gains, freed from the effects of varying food consumption by regression, was subjected to analysis. This procedure was carried out in preference to an analysis of the gains in weight per gram of protein eaten, because, as Crampton (1934) has pointed out, a statistical analysis of the latter ratios includes "in addition to the experimental error, a systematic error which may be of sufficient magnitude to seriously distort the result, and vitiate

the estimate of experimental error and hence any test of significance." The systematic error is of course owing to the fact that no allowance is made in the calculation of the biological values for the amount of food required for maintenance (Crampton and Hopkins 1934a, b). The differences in the corrected gains of the rats made on the fish and beef flesh diets were found to be not quite large enough to be termed significant. The necessary difference between means for P = 0.05 was 7.4 grams; the difference found between the average corrected gain on the beef ration and the highest average corrected gain recorded for the fish ration was 6.7 grams.

Two control diets were fed consisting in the case of diet E of a supplement of 20% vitamin-free casein to the basal diet and in the case of diet F of a supplement of 20% casein plus 5% yeast and 1% liver concentrate (cf. table I). The rats on the latter diet gained 103.4 grams and those on the former diet gained 96.5 grams. Much of this difference in gain may be attributed to difference in food intakes (9.6 g. and 9.8 g. per day on diets E and F respectively). A statistical analysis of the data revealed that the difference could not be termed significant. The necessary difference between the corrected means for P = 0.05, was 10.38 grams; found = 5.06 grams. The biological values calculated for the two diets yielded figures of 1.77 and 1.63 for diets E and F respectively. It therefore appeared that the protein seemed to be utilized just as efficiently, if not more so, by the rats which did not receive the additional B-vitamins in the form of yeast and liver concentrates. On the other hand the higher protein content of diet F would tend to lead to a slightly lower value than that derived from diet E (Mitchell 1924). Further evidence regarding the adequacy of the basal diet is presented in data from trial 3.

FEEDING TRIAL 3

The previous experiment showed that in order to attain the precision necessary to distinguish differences in the biological values of the protein preparations under study, further modifications in experimental conditions had to be made. Initial weight and food intakes were comparable; consequently about the only other main source of variation was the inclusion of males and females in the same groups.

An examination of the results from trial 2 revealed that the values derived from the females were slightly but consistently lower than those obtained from the males despite the fact that the average food intake of the latter was only 249.5 grams as compared to 253.3 grams for the females. Accordingly feeding trial 3 was set up so that adequate numbers of each sex were placed on each diet. Since the values for the four fish flesh products were essentially identical, one (halibut) was chosen at random to be used in further comparative tests. In this way enough rats could be used on each diet to afford a fair test of the differences between groups of rats fed diets containing beef and the fish flesh proteins.

Two changes were made in the basal diet: 10% mazola oil was substituted for an equal amount of beef dripping, and 0.25% each of yeast and liver concentrates was added (see diet C, table I). The latter materials were added to deter-

mine whether or not the increased supply of B-vitamins would affect the efficiency of protein utilization. These concentrates effected the introduction of about 0.28% non-test protein into the diets, or about 3.5% of the total dietary protein. The results are shown in table IV, with additional values for lingcod, lemon sole, white spring salmon, herring, and red snapper, obtained about a year later. The rats were allowed to eat ad libitum.

The data from the animals fed the halibut and beef diets were subjected to the same type of statistical analysis utilized to assess the results of feeding trial 2. The necessary difference between the average gains for P = 0.01 was found to be

TABLE IV.   Biological values of fish and beef flesh proteins.   Initial wt. of rats: 50 ± 2.5 g.
Days on test diets: 28.   Basal diet: C.

Source of dietary protein	Sex	No. of rats	Daily food intake (g.)	Av. gain in wt. (g.)	Gain corr. for food intake (g.)	Gain in wt. per g. protein eaten
Halibut	M	20	10.3	75.5	74.9	3.27
Halibut	F	20	10.5	70.1	68.1	2.98
Beef	M	20	10.2	65.3	66.0	2.86
Beef	F	20	10.1	59.7	61.6	2.63
Lingcod	M	15	8.9	71.7	—	3.60
Lemon sole	M	15	8.9	72.8	—	3.66
White spring salmon	M	15	9.3	77.2	—	3.68
Red snapper	M	15	9.0	78.8	—	3.86
Herring	F	15	8.8	61.4	—	3.18

4.9 and 4.4 grams for the male and female groups respectively. The corresponding differences observed were 9.0 and 6.5 grams and these may therefore be termed highly significant differences.

The remarkably high values obtained for the last five preparations given in table IV are difficult to explain. The figures 3.60 to 3.86 for the males and 3.18 for the females at an 8% protein level are definitely higher than any value previously obtained. The only possible explanation of which the writer can think is that the health and vigour of the rat colony had improved markedly in the interval which had elapsed between the feeding experiments. (In this connection, it might be pointed out that the feeding and care of the rat colony, about one and a half years previously, had undergone some considerable change.) These values are the highest ever reported by this method on proteins of importance in human nutrition. As in trials 1 and 2, the data for lingcod, lemon sole, and white spring salmon agree closely. Such a finding emphasizes the desirability of performing comparative quantitative experiments at the same time or within short time intervals. The value found for the female rats on the herring diet is also higher than that previously found on the halibut and beef flesh diets, although it is in good agreement with the figure obtained from the females on the

diet containing 10% halibut flesh protein in trial 5 (see table VII) which was performed at about the same time.

It is noteworthy that the average biological values for the rats on the halibut (3.13) and beef (2.75) diets are almost exactly the same as those obtained on these diets in the previous experiment in which no yeast or liver concentrates were incorporated (3.15 and 2.80 respectively). Although, indeed, greater gains were observed on the former diets, these could be wholly attributed to greater food intakes as indicated by the similarity in biological values, the calculation of which involves a crude correction for differing food intakes. It would appear from these results that the inclusion of liver and yeast concentrates did not increase the efficiency of utilization of protein, and, further, that the five crystalline vitamins along with the factors present in the flesh and the elements synthesized in the gut on such diets permitted maximal utilization of protein (cf. Beveridge 1946).

Two control diets containing 20% casein as the protein moiety were fed to groups of 10 males each. One diet, G, contained only the amount of yeast and liver concentrates indicated in the test diets. The other diet, H, contained increased amounts of these vitamin supplements, 1% instead of 0.25% of each (see table I). The rats on H ration showed an average gain of 132.0 grams on a food intake of 11.3 grams per day; those on G ration showed an average gain of 112.7 grams on a food intake of 10.3 grams per day. In the latter case, the elimination of data from a rat which gave an abnormally poor growth response (a gain in weight of only 66.8 grams) would have raised the average gain to 117 7 grams. When these data are expressed in the form of gain in weight per gram of protein eaten, the value derived from the data of diet G is 2.14, whereas that from H is 1.98. This result constitutes further evidence that the additions of the yeast and liver concentrates, although increasing the rate of growth under these conditions, do not promote increased efficiency in protein anabolism. The evidence presented permits the conclusion that in the diets employed protein was the principal and almost certainly the only factor which limited protein anabolism.

FEEDING TRIAL 4

Dr. A. T. Cameron, Chairman of the Fisheries Research Board of Canada, suggested that, before publication of these results, another feeding trial at a higher protein level be carried out. This suggestion was adopted and the experiment was performed exactly as in the preceding trial except that crude protein (total dietary N × 6.25) was incorporated at a level of 12%. A test diet containing 12% casein was also included.

Although the difference in the average gains of the male rats on the beef and halibut diets (table V) was shown to be statistically significant by means of the analysis already cited, the difference in gains between the female rats was a little too small to be judged significant. The necessary difference between the corrected average gains of the males for P = 0.05 was 4.9 grams, found: 6.3 grams; the

corresponding data found for the females for P = 0.05, necessary difference: 4.9 grams, found: 4.1 grams.

TABLE V.   Biological values of halibut and beef flesh proteins.   Initial wt. of rats: 50 ± 2.5 g. Days on test diets: 28.   Basal diet: D.

Source of dietary protein	Sex	No. of rats	Daily food intake (g.)	Av. gain in wt. (g.)	Gains corr. for food intake (g.)	Gain in wt. per g. protein eaten
Halibut	M	17	12.4	138.3	135.7	3.32
Halibut	F	16	10.5	95.6	95.7	2.66
Beef	M	17	12.0	126.9	129.4	3.15
Beef	F	16	10.6	91.7	91.6	2.58

The fact that the difference in average gains between the females on the beef and halibut diets could not be shown to be significant was not surprising, since the level at which the test proteins were fed approached and perhaps equalled the level at which maximal growth is elicited.   In this connection it may be recalled that the average gain made by the animals on the adequate control diet H was 132.0 grams, and that brought about by the halibut diet was 138.3 grams. Food intakes were 11.3 and 12.4 grams respectively.   Obviously under such conditions the protein content is not a factor limiting growth and further, as both Osborne and Mendel (1916) and Mitchell (1924) have pointed out, the possibility that an excess of protein above the requirements for maintenance and growth has been consumed, renders any ratio obtained of doubtful value as a measure of the biological adequacy of the particular protein under investigation.

The gains in the six male rats fed the casein diet were compared with those resulting from the corresponding animals on the beef and halibut diets (table VI). Application of the type of statistical analysis to which reference has already been made revealed that for probabilities of 0.05 and 0.01 the differences in the corrected average gains should be 8.1 and 12.3 grams respectively.   The difference in the corrected average gains effected by the casein and beef diets was 11.2 grams in favour of the latter ration and may be termed significant; the corre-

TABLE VI.   Biological values of casein and flesh proteins of beef and halibut.   Initial wt. of rats: 50 ± 2.5 g.   Days on test diets: 28.   Basal diet: D.

Source of dietary protein	No. of rats	Av. daily food intake (g.)	Av. gain in wt. (g.)	Gains corr. for food intake (g.)	Gain in wt. per g. protein eaten
Halibut	6	12.3	136.1	130.7	3.30
Beef	6	12.2	129.7	126.2	3.19
Casein	6	10.8	104.8	115.0	2.89

sponding figure for the casein and halibut diets was 15.7 grams in favour of the fish flesh diet and may be termed highly significant. It therefore appears that casein has a biological value definitely below those of halibut and beef flesh proteins.

EFFECT OF SEX

In a series of experiments to determine the nutritive value of crude protein in animal parts, Hoagland and Snider (1926b) presented evidence showing that larger biological values were obtained from male than from female rats. The

TABLE VII.   Comparison of biological values derived from male and female rats.

Feeding trial	Diets	No. of rats	Sex	Protein level (%)	Av. food intake (g.)	Gain in wt. per g. protein eaten
2.		20	M	8	249.5	3.17
		30	F	8	253.3	2.95
3.	Halibut	20	M	8	289.1	3.27
	Halibut	20	F	8	293.6	2.98
	Beef	20	M	8	285.3	2.86
	Beef	20	F	8	283.1	2.63
5.	Halibut	19	M	10	291.0	3.29
	Halibut	19	F	10	291.0	3.13
4.	Halibut	17	M	12	346.6	3.32
	Halibut	16	F	12	295.1	2.66
	Beef	17	M	12	335.3	3.15
	Beef	16	F	12	295.7	2.58

work of these authors is inconclusive since only a small number of each sex was put on a diet. In many cases the "groups" consisted of only one or two rats. Of fifty comparisons reported, however, all except two showed relatively large margins in favour of the males. The remaining two were about equal. This consistency is all the more remarkable because of the small number of animals utilized in each group. Morgan (1931), on the other hand, has published data from which she concluded that the values derived from the two sexes were essentially the same. An examination of the values cited revealed that of six comparisons, the biological values derived from the males were higher, although only by quite small margins, in five cases; in the remaining instance, the value derived from the female was slightly higher. Morgan's work is inconclusive since only small numbers of each sex were retained on some of the diets.

The results obtained from the males and females of similar initial weights in the present study (trials 2 to 5) are shown in table VII. Despite the fact that the food intakes of the males and females were almost exactly the same in

trials 2 and 3, the biological values derived from the former animals were higher in every case. In trial 4 the males ate significantly more than did the females and the margins by which the values obtained from the males exceeded those from the females was much higher than in the previous trials. It may be of interest to note the consistency in the differences in biological values derived from the two sexes at similar protein levels. For example in trial 2 the difference was 0.22, in trial 3 on the halibut diet, 0.29, and on the beef diet, 0.23. At the 12% protein level in trial 4 the differences were not quite so consistent being 0.66 and 0.57 for the halibut and beef flesh diets respectively.

The difference between the corrected average gains of males and females was in every case shown to be highly significant. For example the differences between the corrected means of the gains made by the male and female rats on the beef diets were, at the 8% protein level, 4.91 grams, and at the 12% protein level, 22.41 grams. For P = 0.01 the necessary differences were found to be 3.96 and 8.85 grams respectively.

These findings appeared to indicate that the male rats actually utilized the protein of their ration more efficiently for growth. It was felt that such an unexpected result warranted fuller investigation and, in the course of some later work in determining the biological value of certain proteins, a group of 19 males and females were paired-fed on a diet containing halibut flesh protein at a level of 10%. The animals were very carefully paired, the initial weights of the paired rats being within 0.5 grams of each other and usually within 0.2 grams. The test diet was the same as diet C (table I), except that the test protein was incorporated at the level just now indicated. The paired feeding was highly successful. Average gains of 95.8 grams and 91.1 grams were recorded for the males and females respectively. The necessary difference for P = 0.05 was found to be 3.50 grams and for P = 0.01, 4.77 grams. The observed difference, 4.70 grams, therefore just fails to be of sufficient magnitude to be judged highly significant but may be termed significant. The pertinent question as to whether or not the composition of the new tissue of the males and females differed might very well be raised at this point. Data regarding this are available from feeding trial 2. Although the average percentage nitrogen composition differed by only 0.005% (2.793% and 2.788%), and by inspection seemed not significantly different, an analysis of variance was performed on the series. The necessary difference for P = 0.05 was found to be 0.226. The observed difference was therefore definitely not significant. Furthermore, an examination of the data obtained by the nitrogen retention method of McCollum and Shukers (cf. McCollum and Simmonds 1929) in trial 2 revealed that the average biological value derived from the males was 54.9, and that from the females, 51.1. This result substantiates the evidence previously presented that male rats, under the conditions herein described, utilize the dietary protein more efficiently for growth than do the females.

In figure 1, shown in the section concerned with the effect of length of feeding time on biological value, it is apparent that the efficiency of utilization of protein in the female rats does not fall below that of the males until about half way

through in the 28-day test period. This phenomenon roughly corresponds with the marked flattening of the growth curve of the females and it would appear that the food intake of the female does not decrease correspondingly with decrease in growth rate. (The somewhat higher value obtained on the eighth day from the females on the diet containing 10% protein is due to a slightly higher food consumption during the first few days than was evidenced by the males).

The differences in food intake and biological values observed in trial 4 are typical of the work and confirm the findings reported by Hoagland and Snider (1926 a, b). The latter attributed their result to the fact that, since the male

FIGURE 1. Relationship between length of time of feeding and efficiency of protein utilization by male and female rats.
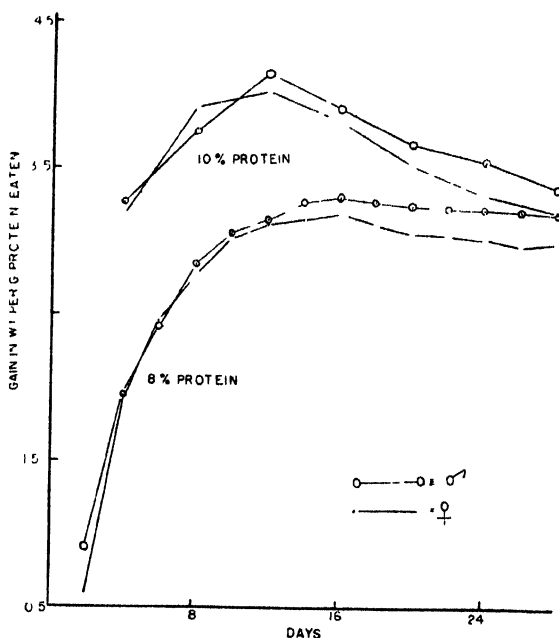
rats ate more, they required a smaller proportion of their food intake for maintenance than did the females. On the other hand, as Morgan (1931) has pointed out, a neutralizing tendency would be the increasing maintenance requirements of the larger animals.

The steeper growth curve of the males is a well known phenomenon and any change which brings about conditions more nearly optimal for growth will permit the male rats to attain a growth rate more nearly normal and accentuate the difference in values shown by the two sexes, (compare results on the 8 and 12% protein diets, table VII). Paired feeding naturally will depress the curve and consequently differences between biological values derived from males and females will be correspondingly more difficult to discern since the potentiality for growth of the female will not have been altered to the same extent as that

for the male. The same holds true when proteins of low biological value are fed to the two sexes as Morgan (1931) did. It is, therefore, not surprising that the latter did not observe marked differences in the ratios obtained for the two sexes. The flatter growth curve of female rats is fairly evident in the period shortly after weaning and, since the evidence presented here indicates that the dietary protein is not so efficiently utilized as by male rats, it would appear that the food intake of the female does not decrease to the same extent as does the growth rate. A number of possible explanations for the sex difference in protein utilization may be mentioned: (1) a greater proportion of protein may be catabolized by the females; (2) males may digest and absorb a slightly larger percentage of ingested protein; (3) the proportion of essential amino acids required to promote maximal growth in females may differ from that required for males.

EFFECT OF LENGTH OF TEST PERIOD

In figure 1 is shown the relationship between the calculated biological values and times for the males and females of feeding trials 2 and 5. Boas-Fixsen (1935) published a review in which she asserted that the miminal length of feeding time required for accuracy was 60 days. The data presented in figure 1 indicate that in about 16 days the protein efficiency ratios reach a peak and then assume fairly constant values which, of course, diminish gradually with time. The value for the males on the 8% protein diet decreased from a peak of 3.31 at 16 days to 3.21 at 28 days. The corresponding values for the females were 3.20 and 3.00. The curve obtained from the animals on the 10% protein diet differs to some extent from that obtained from the animals fed the diet containing the lower level of protein. It may be worth while to recall that the animals on the former diet were paired-fed although some slight divergence in food intake occurred during the first few days. The latter diet was fed as previously described on a slightly restricted basis. It may be concluded that the results obtained in this investigation indicate that an experiment of 60 days' duration is unnecessarily long and wasteful of time and material; whereas 21 to 28 days' feeding time appears to be adequate in determining the relative effectiveness of proteins for growth.

DISCUSSION

Because of the many variables involved in the determination of the biological value of proteins it is difficult to compare adequately results obtained from other laboratories. Lanham and Lemon (1938) have reported values for a number of fish flesh preparations only one of which was treated in the present investigation. The values found by them varied from 1.88 to 2.23. Their preparations, however, were made in a different way (acetone extraction); protein level utilized was 9%; and the feeding period was 10 weeks. It is interesting to note, however, that these workers also found that the crude proteins of fish flesh were definitely superior to those of beef flesh. In an extensive study on the nutritive value of crude protein in animal parts, Hoagland and Snider (1926b) obtained values for beef muscle of 3.15 for male rats and 3.00 for females. The initial weight of the

rats was about 40 grams, protein level (N × 6.25) 10%, and feeding period 30 days. These conditions are not exactly the same as, but approximate those employed here. The biological values, considering the somewhat different conditions, agree fairly well. The same workers, in an earlier paper (1926a) reported a value of 1.98 for casein obtained under similar conditions with male rats. The value of 2.89 found in the present work, even when the differing conditions are taken into account, is widely divergent. Hoagland and Snider did not demonstrate the adequacy of their basal diet, and the author suggests that the foregoing data indicate that it was inadequate. The fact that figures of the same order of magnitude were obtained at both laboratories on beef muscle may be explained by the probable presence in the beef of the factors absent or deficient in the basal diet. The casein tested by Hoagland and Snider was a "highly purified product" and consequently would contain negligible amounts of the B-vitamins; this preparation would not, therefore, make up any deficiency in the basal diet. Under such circumstances the food intake would be low and a low biological value would be obtained. Both these features exist in the report under consideration. This situation on the other hand would also happen when a protein of low biological value was being tested. However, the result of the present study indicates that casein is a protein of relatively high biological value and hence the adequacy of the basal diet used by Hoagland and Snider must be questioned.

SUMMARY

1. The biological values of the crude flesh proteins of lingcod, halibut, lemon sole, white spring salmon, red snapper, herring, beef and of egg albumin have been determined using rats of initial weight 50 ± 2½ grams, a feeding period of 28 days, and a protein level of 8%.

2. The gains brought about by the fish flesh proteins corrected for varying food intake were shown to be significantly greater than those brought about by the beef flesh proteins. The biological value of egg albumin was of the same order of magnitude as that of the beef flesh proteins.

3. Under the same conditions listed in point (1) but at a protein level of 12%, the biological values of the proteins of halibut flesh, beef flesh, and casein were compared. The biological values of the two flesh proteins were shown to be significantly greater than that of casein, and that of halibut significantly greater than the value derived from the beef flesh proteins.

4. Evidence has been submitted to show that male rats even at similar food intakes utilize the protein more efficiently for growth than do females.

5. The graph of efficiency of protein utilization against time revealed that feeding periods of 21 to 28 days are entirely adequate in determining the biological value of proteins.

to Messrs. O. C. Young and E. P. Sidaway for their assistance in cooking and drying the first of the flesh preparations, and to Misses Phyllis Boyce and Lucille Gardner for the feeding and care of the experimental animals and to the latter for performance of some of the technical work.

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Sulphur Distribution in Fish Flesh Proteins

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ABSTRACT

The sulphur distributions in the crude flesh proteins of lingcod, halibut, lemon sole, and white spring salmon have been determined. The proteins of the species examined proved to be excellent sources of methionine and the figures obtained for each amino acid agreed closely amongst the different species.

Many and diverse properties of proteins in nutrition have been brought to light in recent years. Investigators have demonstrated injurious effects of protein deficiency on the healing of wounds, resistance to infections, chloroform liver injury, etc. (cf. Elman 1944, Stare, Hegsted and McKibbin 1945, Co Tui 1946, and Stone and Davidson et al. 1945). These properties are in the main a function of the constituent essential amino acids. On the other hand Woolley's (1946) report, in which he describes the existence of a peptide or peptide-like substance associated with proteins which accelerates growth, indicates that in so far as this function is concerned, amino acids of the protein may not be wholly responsible for the effects observed. This factor has been termed strepogenin; at time of writing, however, Woolley's work is difficult to assess because of the meagre information given in his reports. For example, in describing the effect of the newly discovered substance on the growth rate of mice, he neglects to report food intakes. The effect observed may, therefore, merely be one of palatability. Until further evidence is forthcoming it may be assumed that the nutritional properties of proteins are in the main a function of the essential amino acids contained therein.

It is, therefore, highly important that data be obtained by the best available methods on the important protein foods. The present report contains the values derived for methionine and cystine in lingcod (*Ophiodon elongatus*), halibut (*Hippoglossus stenolepis*), lemon sole (*Parophrys vetulus*), and white spring salmon (*Oncorhynchus tschawytscha*). Recent publications describing certain interesting physiological properties of these substances such as their effect on liver fat levels (Beeston and Channon 1936, Tucker and Eckstein 1937), their ability to prevent chloroform injury in hypoproteinemic dogs (Miller, Ross and Whipple 1940) and the transfer of the essential methyl group of methionine to form choline *in vivo* (Du Vigneaud *et al.* 1940) emphasize the desirability of acquiring analytical data on these amino acids in proteins of nutritional importance.

51

1

Samples of the fish flesh separations used in determining their biological values as described by Beveridge (1947) were utilized for the analyses presented in this paper. Fat was extracted by allowing 300 g. of meal to soak for 48 hours in 3000 cc. 3:1 alcohol-ether solution. The materials were then filtered off and extracted with ether in a soxhlet apparatus for about 24 hours. The air-dried substances were extracted with three successive 3000 cc. portions of boiling water. The lingcod and lemon sole preparations could not be filtered efficiently in this

TABLE I. Proximate analyses of crude fish flesh proteins.   Nitrogen and sulphur figures are based on ash- and moisture-free material.

Source of protein	Moisture (%)	Ash (%)	Nitrogen (%)	Sulphur (%)
Lingcod	3.21	1.31	16.51	1.213
Halibut..................... ...	2.29	0.71	16.50	1.183
Lemon Sole.....	3.68	1.20	16.62	1.177
White spring salmon.......	2.95	0.67	16.60	1.166

TABLE II. Sulphur distribution of fish flesh proteins.   In no case was inorganic sulphur present in determinable amount.

Source of protein	Methionine (%)		Cystine (%)		Sulphur by summation (%)	Sulphur by oxidation (%)	Recovery (%)
	Author	Others	Author	Others			
Lingcod......... ..	3.82		1.46		1.211	1.213	99.8
Halibut............	3.66		1.47	1.45*	1.179	1.183	99.7
Lemon sole........	3.72		1.40		1.173	1.177	99.7
White spring salmon..... . ..	3.64	3.78**	1.36	1.27*	1.146	1.166	98.3

*Pottinger et al. 1939.
**Beach et al. 1943.

procedure and were subjected to centrifugation to get rid of the hot water. The meals were dried in a vacuum oven at 40 to 45° C. and placed in stoppered bottles.

In the proximate analyses of crude fish flesh proteins (table I), the moisture of the protein preparations was estimated by drying samples to constant weight *in vacuo* over phosphorus pentoxide; sulphur was determined as sulphate after fusion with sodium peroxide and sodium carbonate. Nitrogen was determined by the macro-kjeldahl method.

For the sulphur distribution (table II), analysis was carried out in triplicate or quadruplicate by the Kassell and Brand modification (1938) of Baernstein's method (1936a). The use of a mercury seal between the digestion flask and

reflux condenser, as recommended by the former workers, was found to be unnecessary. Although the data reported from the literature were obtained by different methods, the agreement is good. Representative values for methionine and cystine in casein, beef muscle and egg albumin obtained by the same methods as utilized by the author are given in table III.

An examination of tables II and III reveals that egg albumin contains definitely more methionine and slightly more cystine than any of the proteins cited. This material probably has the highest methionine content of any of the common protein foods. In their excellent monograph on the amino acid composition of

TABLE III. Methionine and cystine contents of casein, beef muscle and egg albumin.

Protein	Methionine (%)	Cystine (%)	Reference
Casein (Labco)...........	3.15	0.53	(1)
Casein (Labco)...........	3.17	0.39	Kassell and Brand 1938.
Casein (Harris).........	3.31	0.29	Baernstein 1936 a, b.
Beef muscle........... ..	3.19	0.97	(1)
Egg albumin.....	5.07	—	Baernstein 1936 a.
Egg albumin...........	5.23	1.78	Kassell and Brand 1938.

(1) These values were reported in a paper by Beveridge, Lucas and O'Grady (1945) uncorrected for moisture and ash content. They are presented here on a corrected basis.

proteins and foods, Block and Bolling (1945) list no other protein with as high a content of methionine as egg albumin. As a class, fish flesh proteins appear to be the next best source of this essential amino acid. The figures herein reported are higher than those obtained in the same way on casein and beef muscle. This finding is probably partly responsible for the relatively high biological values obtained with fish muscle proteins (cf. Beveridge 1946) for it is well known that the sulphur-containing amino acids are the main limiting factors in casein for the promotion of growth (Mulford and Griffith 1942).

SUMMARY

The sulphur distributions of the crude flesh proteins of lingcod, halibut, lemon sole, and white spring salmon have been determined.

Within the limits of experimental error all the sulphur present was accounted for in the form of methionine and cystine, thus precluding the existence of appreciable quantities of other sulphur-containing compounds.

The methionine and cystine values found in the different species varied from 3.64 to 3.82% and 1.36 to 1.47% respectively.

These figures indicate that of the proteins important in human nutrition, except for egg albumin, the crude proteins of fish constitute the best source of methionine.

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PACKAGING

VI. THE RELATIVE MERITS OF VARIOUS TYPES OF BAG CONSTRUCTION IN PRODUCING WATER-VAPOUR RESISTANT PACKAGES

BY C. G. LAVERS

PACKAGING

VI. THE RELATIVE MERITS OF VARIOUS TYPES OF BAG CONSTRUCTION IN PRODUCING WATER-VAPOUR RESISTANT PACKAGES[1]

By C. G. Lavers[2]

Abstract

Water-vapour penetration was measured on pouch, flat, wedge, and square liner bags fabricated from Reynold's Metal A-51, 300 M.S.A.T. "Cellophane" coated 40 lb. per ream with a flexible wax composition, 55 lb. laminated bleached glassine, and 300 M.S.A.T. Cellophane. The bags were closed, where the material permitted, by heat-, glue-, and pressure-sealing, and by folding with or without tin-tie closures.

When Reynold's Metal A-51 or waxed Cellophane was used, excellent water-vapour resistance could be achieved with any of the bag types investigated, and a folded closure was as efficient as a heat-seal. With all materials except 300 M.S.A.T. Cellophane, bags made with glue were almost as good as those with heat-sealed construction. Unwaxed Cellophane or glassine provided more protection when the simpler forms of bags (pouch) were used. With unwaxed Cellophane, heat-sealing appeared to make a better liner than the use of glue, and a heat- or glue-sealed closure was superior to a double fold.

Introduction

An important factor in the packaging of many foods is the prevention of the passage of water-vapour either into or out of the package. Many flexible water-vapour barriers have been developed for this use. The water-vapour resistance of many of these barriers has already been assessed (1, 2), and it has been shown that, for products not likely to rupture the barrier, the best method of applying a water-vapour barrier is as a liner inside a carton (1). While the pouch type liner bag has generally been assumed to provide the greatest resistance to passage of water-vapour, little definite information is available on the relative merits of various types of bag construction. The purpose of this paper is to compare the water-vapour resistance of liner bags made in the basic commercial styles.

Materials and Methods

The names applied to various methods of forming bags are not constant from manufacturer to manufacturer, therefore the styles of bags tested and the names applied for the purpose of this study are illustrated in Fig. 1. The 'pouch' bag is the simplest to make, having only a seam at either side, and there is the least possible opportunity for water-vapour transmission through seams and seals. The 'flat' bag has two seams, one along the side, and another at the bottom; the junction of the side and bottom seams is a

PLATE 1
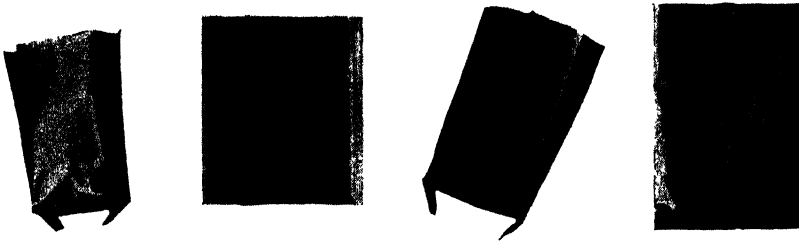
POUCH   FLAT

WEDGE

SQUARE   SQUARE

FIG.1

FLAT   WEDGE

FIG.2

FIG. 1.   *Construction of the various types of bags studied.*
FIG. 2.   *Some methods of closing bags used in this study.*

possible point of entry for water vapour. The 'wedge' type bag shown in Fig. 1 has a single side seam, gussets, and a wedge closure (Fig. 2) at the bottom. The additional folds in this type of bag might be expected to cause higher rates of water-vapour transmission in it than in the previous types. Glue-sealed 'square', or self-opening bags, shown in Fig. 1, were made with both the common grocery bag style of bottom, and with a 'flat' seal (Fig. 2) on the bottom. The flat bottom seal should improve the water-vapour resistance of this type of bag over that of the wedge style of bag unless the material used is sensitive to the added creasing required to make it square when opened. Square bags made using heat-sealed construction were necessarily of the flat bottom closure style.

Besides the closures illustrated in Fig. 2, unsealed single and double fold closures, some with and some without a tin-tie were tested. When folded closures are used special care must be taken to see that the box is well filled so the top of the carton will prevent the fold from opening. In the course of the study, pressure sealed bags were also examined. Only heavily waxed material could be used for this purpose. All seams were heat-sealed except the final closure, which was made by running a dull pencil point across the mouth of the bag, causing the waxed surfaces to stick together.

Four materials with various characteristics were used to make the liners for this investigation. These were: Reynold's Metal A-51 (kraft paper laminated to metal foil coated with Butvar), 300 M.S.A.T. "Cellophane" coated 40 lb. per ream with a flexible wax composition, 55 lb. laminated bleached glassine (coated on one side with a heat sealing composition), and 300 M.S.A.T. Cellophane. When Reynold's Metal A-51 was used to make glue-sealed bags it was necessary to reverse the material and place the kraft on the inside of the bag and the heat-sealing surface outside.

The combinations of materials, bag styles, and closures tested are given in Table I.

The method of measuring water-vapour transmission was the same as that used in a previous investigation (1). The materials were fabricated into liners of suitable size to fit inside a light chipboard carton, 4 by $2\frac{1}{4}$ by $1\frac{5}{16}$ in. (opening end, $2\frac{3}{4}$ by $1\frac{5}{16}$ in.). The liner was opened, inserted into a carton, and partially filled with sawdust; then 73.5 gm. of anhydrous calcium chloride in a perforated P.T. Cellophane bag was added; this bag was surrounded by sawdust and the remainder of the liner was filled with sawdust; the liner and carton were then closed. Six filled packages were used to test each type of liner bag, and six packages without calcium chloride and sawdust were used to estimate the sorption of water-vapour by the packaging materials. The filled packages and dummies were placed in a cabinet operating at 95° F. and 100% relative humidity (vapour-pressure differential, approximately 42 mm. of mercury), and moisture gain was determined by weighing each package at weekly intervals for four weeks.

TABLE I

Water-vapour transmission (gm. per week) of various types of liner bags

(Standard error for mean transmission rates, 0.32 gm. per week)

Construction and type of bag	Closure	Packaging material			
		Reynold's Metal A-51	300 M.S.A.T. Cellophane coated 40 lb./ream wax composition	55 Lb. laminated bleached glassine, coated with a heat-sealing composition	300 M.S.A.T. Cellophane
Glue-sealed	**Glue-sealed**				
Pouch	Flat	0.26	—	1.29	2.86
Flat	Flat	0.22	—	1.98	4.91
Wedge	Wedge	0.26	—	1.75	5.78
Square, bottom sealed as for flat bag	Wedge	0.22	—	2.25	3.74
Square, bottom sealed as in a grocery bag	Wedge	—	—	2.33	3.74
Heat-sealed	**Heat-sealed**				
Pouch	Flat	0.0	0.29	1.24	1.46
Flat	Flat	0.17	0.47	1.39	2.53
Wedge	Wedge	0.34	0.43	1.70	2.78
Square, bottom sealed as for flat bag	Flat	0.0	0.44	3.43	3.78
	Wedge	0.0	0.83	2.92	4.16
	Unsealed				
Pouch	Single fold, tin-tie	0.0	—	—	—
	Double fold, tin-tie	0.0	—	—	—
Square, bottom sealed as for flat bag	Double fold, flat	0.19	0.53	3.24	4.68
	Double fold, wedge	0.21	0.82	3.10	4.69
	Pressure sealed				
	Double fold, flat	—	0.60	—	—
	Double fold, wedge	—	0.56	—	—
	Single fold, flat	—	0.62	—	—

Results

Water-vapour transmission rates of the various types of bags tested are shown in Table I. These values were calculated by the method used in a previous study (1). The standard error for the mean transmission rates reported in the present study was of the same order of magnitude for all the different bag types. The average standard error was 0.32 gm. per week. For all types of bags the protection provided by the different materials in decreasing order of efficiency was: Reynold's Metal A-51, 300 M.S.A.T. Cellophane wax-coated 40 lb. per ream, 55 lb. laminated bleached glassine, and 300 M.S.A.T. Cellophane. This agrees with results obtained in a previous study (1).

The various types of liner bags made from Reynold's Metal A-51 did not differ significantly in behaviour. However, the transmission of glue-sealed bags was generally slightly higher than that of those made with heat-sealed construction, and the heat-sealed wedge bag appeared slightly inferior to the

other types.   The water-vapour transmission of unsealed bags (folded closures), with or without a tin-tie, did not differ markedly from that of bags that were heat-sealed, but the addition of the tin-tie to a folded closure improved the water-vapour resistance somewhat.

Although no significant differences occurred in the water-vapour resistances of the various types of bags made of wax-coated Cellophane, the pouch type bag appeared to be slightly superior.   Little difference in transmission was expected between types made with this material because the heavy wax coating when melted in the heat-sealing operation would flow into places around folds in the seal that might be left open when other materials are used. Unsealed folded closures were as good as those that received the added treatment of pressure sealing.   This would probably not be true, however, if the fold was not held closed by the outer carton.

For any one type of bag little difference was noted between glue- and heat-sealed construction with liners made of laminated glassine.   There was, however, a tendency for the efficiency of the bag to decrease as the complexity of construction increased, i.e., from pouch to flat to wedge to square.   This was attributed to the combined effect of increased difficulty in sealing and the added folding of the material required in making the more complex bags. Glassine, being a relatively brittle material, develops pinholes easily when folded.   Square, glue-sealed liners with the bottom sealed as in a grocery bag were as good as those with a flat seal on the bottom; and the water-vapour resistance of bags with a folded closure was equal to that of bags closed by heat-sealing.

Unlike the bags made of laminated glassine, heat-sealed Cellophane bags in the pouch, flat, and wedge styles were superior to those made with glue. This was probably due to the greater spring-back that occurs when Cellophane is folded.   Cellophane is a very thin material, not readily creased, and it is difficult to obtain a perfect glue-seal (especially at corners) unless true surfaces are used to hold the material in place until such time as the glue has set.   As with glassine, the more complex types of bags, whether heat- or glue-sealed, were generally less efficient water-vapour barriers.   Equal protection was provided by square, glue-sealed bags with grocery or flat style bottoms. Folded closures on Cellophane bags were inferior to heat seals, likely because the thin springy nature of this material caused some opening of the fold even though the bags were well filled and held shut by the outer container.

Discussion

When the results in Table I are considered, it must be remembered that all bags were carefully made in the laboratory, and great care would be required to produce bags of equal quality on a commercial scale.   For example, a square bag with a grocery type bottom as used in this experiment would be sealed more completely than the same product from most bag making machines at present in use.   However, once the plant process has been perfected it

should be possible to make bags in any style superior to those used in this study. Nevertheless the results given here should represent the relative effectiveness of the various bag types, materials, and closures.

Fairly heavy materials, like Reynold's Metal A-51, and heavily waxed sheets (flexible wax composition) that are not sensitive to creasing can be formed into bags with high water-vapour resistance regardless of the type of bag used, and a folded closure can be as efficient as a heat-seal. However, materials that are sensitive to creasing, such as glassine, are best utilized for only the simpler types of bags. With materials like Reynold's Metal and glassine, bags sealed with glue are almost as good as those made with heat-sealed construction.

Thin springy materials, like unwaxed Cellophane, provide more water-vapour resistance when the simpler forms of bags (pouch) are used, and a heat- or glue-sealed closure is superior to a double fold. Heat-sealing rather than the use of glue appears to make a better liner with this packaging material.

The above results indicate that it is generally desirable to keep bag construction as simple as possible if the most protection from loss or gain of water vapour is to be expected. However, the bags of simple construction are often more difficult to use in small scale commercial or home operations. For these uses, the more complex bags may be satisfactory for packaging many products provided suitable materials are employed.

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PRECISION OF ASSESSMENT OF PALATABILITY OF FOODSTUFFS BY LABORATORY PANELS

II. SALTINESS OF BACON[1]

By J. W. Hopkins[2]

Abstract

Numerical ratings of the salty taste of freshly cooked portions of 80 pieces of Wiltshire-cured Canadian bacon by each member of a panel of 23 judges are analysed statistically, with results in general qualitative agreement with those previously reported for other palatability tests made in the same laboratory. Single assessments were subject to considerable random variation superimposed upon wide differences between individuals in respect of both tolerance and sensitivity. Nevertheless, a significant element of correlation made possible reproducible results, although it is calculated that to discriminate differences of the order of 5% on the organoleptic scale would have required 35 and 62 judges for intra- and inter-panel comparisons, respectively. The preferred degree of saltiness corresponded to a sodium chloride content of the cooked bacon of about $4\frac{3}{4}$% in the absence, and of roughly 4% in the presence of $2\frac{1}{2}$ parts per thousand of sodium nitrate.

Introduction

In an earlier paper (3) the writer distinguished between 'grading' (absolute) and 'analytical' (relative) assessments of the palatability of foodstuffs by panels of judges, and described the results of statistical analyses of ratings of butter, dried eggs, dried milk, and ration biscuits made in these laboratories. These analyses included a numerical investigation of the judging characteristics of individuals by computation of the correlation coefficients and regression equations relating their assessments to the average of those of all other members of the same panels. A parallel study has since been made of assessments of the saltiness of cooked bacon, the outcome of which is reported now.

Data

Data for this study consisted of numerical ratings of the saltiness of freshly cooked portions of 80 different pieces of Wiltshire-cured Canadian bacon by each member of a panel of 23 judges, 17 male and 6 female, recruited from the scientific, technical, and administrative staff of the Divisional laboratories. Chemical analysis of samples of the cooked material indicated contents of sodium chloride ranging from about $4\frac{1}{2}$ to 9%, of sodium nitrate from 1/20 to $\frac{1}{4}$%, and of sodium nitrite from 15 to 55 parts per million. Each judge tasted samples from all 80 pieces in groups of four and at the rate of eight per day. Subjective appraisals of saltiness were expressed numerically in accordance with the convention currently adopted in these laboratories. In this, which

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differs from the one previously used (3), zero represents the ideal in the estimation of the judge, and excess or deficiency of the flavour in question is rated on an integral scale of $+ 5$ to $- 5$.

Results

An analysis of the variance (2) of the 1840 foregoing individual assessments gave mean squares of 54.743 between judges (averaged over all samples), of 11.783 between pieces (averaged over all judges), and of 1.133 for the residual fluctuations of individual ratings. The standard deviation of an average rating appropriate to intra-panel comparisons of pieces was thus $\sqrt{1.133/23}$, i.e., ± 0.22. To attain the 5% level of statistical significance therefore, a difference in the average ratings of two pieces would have to be at least $1.96\sqrt{2 \times 1.133/23}$, namely, ± 0.61. To reduce this 'necessary difference' to ± 0.50 would have required a panel of 35 judges.

From the mean squares listed in the preceding paragraph the underlying 'population variance' of judges' averages may be estimated (1) at $(54.743 - 1.133)/80$, corresponding to a standard deviation of ± 0.82, although this figure, which involves one component based on only 22 degrees of freedom, is subject to correspondingly more uncertainty than that for the residual variance, for which 1738 degrees of freedom were available. From this estimate, therefore, it would have to be inferred that to be regarded as statistically significant at the 5% level, a difference in average ratings of samples assessed by two separate panels of the same size and variability as the one here considered would have to amount to at least $2.069\sqrt{2 \times (0.6701 + 1.133)/23}$ i.e., to ± 0.82. Panels of the order of 60 members each would therefore be estimated as required to reduce this inter-panel necessary difference to ± 0.50. If each judge made two independent assessments of samples from the same piece, this number would be reduced to rather more than 40, but only at the cost of increasing the total number of man-hours devoted to assessment in the ratio of about 85 to 60, as well as of entailing a corresponding increase in the quantity of test material and in the labour of preparation.

As in all the four cases previously considered, there were pronounced differences in personal preference, the average assessment of the saltiness of all 80 pieces by individual judges ranging from $+ 3.24$ to $- 0.54$, i.e., from considerably over to slightly under the desired amount. The average assessment by the panel as a whole was $+ 1.20$. Table I shows the deviation of the average for each judge from that of the remainder of the panel, and also lists the correlation and regression coefficients relating the assessment of single samples by each judge to the average assessment of samples of the same piece by the rest of the panel. As the average numerical assessment of the various pieces ranged only from $+ 2.70$ to $- 1.13$, while the irregular fluctuation of single assessments has already been noted as giving rise to a mean square deviation of 1.133, generally high coefficients of correlation of individual with group assessments could not be expected. In fact the highest obtained was

TABLE I

STATISTICAL CHARACTERISTICS OF INDIVIDUAL JUDGES' ASSESSMENTS

Judge	Average deviation from mean of all others	Coefficient of correlation with means of all others	Coefficient of regression on means of all others
No. 1 (male)	− 0 08	.57***	0.85
No. 2 (female)	− 0.31	.59***	1.32
No. 3 (male)	− 0.53	.63***	0.78
No. 4 (male)	− 0 26	.45***	0.39
No. 5 (male)	+ 0 89	57***	0 90
No. 6 (male)	+ 0 84	.69***	1 06
No. 7 (male)	+ 1.07	33**	0 52
No. 8 (female)	− 0.38	.41***	0 69
No. 9 (male)	− 0.89	.11	0.02
No. 10 (female)	+ 0.20	.56***	0 89
No. 11 (male)	+ 0 97	.70***	1 72
No. 12 (female)	− 0.12	.64***	0 82
No. 13 (male)	− 0 33	.51***	0 55
No. 14 (female)	− 1 06	47***	0 60
No. 15 (male)	+ 0 45	.64***	0 92
No. 16 (male)	− 0 02	.66***	1 52
No. 17 (female)	− 0 47	46***	0 64
No. 18 (male)	+ 0.26	.62***	1 18
No. 19 (male)	+ 2.13	43***	0.93
No. 20 (male)	− 0.17	.70***	1 18
No. 21 (male)	− 1.11	15	0 37
No. 22 (male)	− 1 82	32**	0 59
No. 23 (male)	+ 0 78	.62***	1 24

** *Attains 1% level of statistical significance.*
*** *Attains 0.1% level of statistical significance.*

0.70. However, eight of the judges had coefficients in excess of 0 60, which in the circumstances are regarded as reasonably satisfactory, and those of only two were not statistically significant.

The range of individual sensitivity indicated by the regression coefficients of Table I is greater than that provided by the previously reported (3) assessments of over-all palatability. Judge No. 20, with an average deviation from the mean of all others of only − 0.17 unit, a correlation coefficient of 0.70, and a regression coefficient of 1.18, conformed most closely to the average reaction of the group as a whole. No. 11, who had an average deviation of + 0.97 and correlation and regression coefficients of 0.70 and 1.72 was the most sensitive to variations in saltiness, to which on the other hand Nos. 9 and 21 appeared to be very largely (the former almost completely) indifferent. Nos. 19 and 22, with average deviations from the mean of all other judges' assessments of + 2.13 and − 1.82, respectively, provided the individual extremes of aversion to and tolerance for saltiness. All the individuals specified were males, but the results as a whole were not indicative of any statistically significant average difference between the sexes in respect of either tolerance or sensitivity.

The present results differ from the majority of organoleptic assessments in that they may be appropriately related to specific chemical constituents of the cooked meat, and in this way it was found that the coefficients of partial correlation of the average assessment and the sodium chloride and nitrate contents reported for the various pieces were + 0.62 and 0.25, respectively, the latter attaining the 5% level of statistical significance. The small variations in sodium nitrite recorded were without demonstrable effect on the assessment. It would be unjust to attribute the moderate level of even the first of these correlation coefficients entirely to inconsistency of the judges or masking effects of other flavours, as it was evident that the chemical determinations were themselves subject to appreciable sampling discrepancies.

The average assessment for each piece obtained from the panel as a whole was related to sodium chloride and nitrate content by the regression equation of least squares:

$$Assessment = -1.78 + 0.375 \ (NaCl \ per \ cent)$$
$$+ 0.090 \ (NaNO_3 \ per \ mille) \pm 0.45.$$

As no significant reduction of the residual variance resulted from inclusion of the quadratic term in NaCl, it was inferred that the relation was sensibly linear over the range considered.

Owing to its rather limited range of saltiness, the present series of observations is not very informative in respect of the variance of individual assessments at different average preference levels. The inter-judge standard deviations for all samples receiving average assessments between 0 and 1, between 1 and 2 and between 2 and 3 were ± 1.29, ± 1.18, and ± 1.43, respectively. The last of these is significantly larger than either of the other two, and thus provides some indication of slightly greater divergence in the assessment of moderately unpalatable samples. Additional data are, however, required to investigate this point adequately.

Conclusions

These results for the specific attribute of saltiness of bacon are in qualitative agreement in four respects with those already reported (3) for general palatability of certain other foodstuffs. First, they indicate that single assessments by unselected judges were characterized by considerable random variation but also by a demonstrable element of correlation, making possible reproducible differentiation between samples by a panel of adequate size. Second, they again provide evidence of persistent differences in the preference level of individuals, necessitating substantially more judges for a specified degree of reproducibility in inter- than in intra-panel comparisons. Third, individual judges again exhibited a wide range of sensitivity as well as of tolerance. Fourth, there was some further indication of greater diversity in individual assessments of moderately unpalatable samples.

The significant degree of correlation of the panel assessments with the sodium chloride and nitrate content of the test material is a satisfactory

feature of the present findings. From the regression equation given in the preceding section it may be computed that the degree of saltiness to the taste preferred by this panel would be obtained from a sodium chloride content of about $4\frac{3}{4}\%$ by weight of the cooked bacon in the absence of sodium nitrate, and of roughly 4% when $2\frac{1}{2}$ parts per thousand of sodium nitrate was also present.

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THE EFFECT OF METHOD OF COOKING ON THE MOISTURE CONTENT OF CANNED PRE-COOKED POULTRY MEAT

By D. MacDougall and N. E. Gibbons

THE EFFECT OF METHOD OF COOKING ON THE MOISTURE CONTENT OF CANNED PRE-COOKED POULTRY MEAT[1]

By D. MacDougall[2] and N. E. Gibbons[3]

Abstract

Moisture content of the meat and solid content of the broth from open- and pressure-cooked chicken and fowl were investigated during cooking and subsequent processing. During cooking, pressure-cooked meat lost more moisture than open-cooked meat, but during canning and separating the reverse was true. For these reasons, approximately 4 oz. of open-cooked meat, as compared with 3.6 oz. of pressure-cooked meat, must be canned to obtain 3.5 oz. of meat in the finished product. During cooking there was a greater loss of moisture from dark than from light meat. Under all the cooking conditions used the loss of weight from fowl during processing and separation was greater than that from chicken meat. The loss in weight of meat during canning and processing is mainly due to moisture changes rather than the loss of solids.

Introduction

Canadian regulations (1) state that canned poultry to be sold as 'jellied pack' must have a solid meat content of not more than 55% and not less than 50% of the final pack. Poultry canners who used the open-cook method were originally led to believe that, if 3½ oz. of solid meat were to be obtained from a 7-oz. can after retorting and separating, it was necessary to place 3¾ oz. of cooked meat in the can. Both canners and poultry inspectors found that the loss of weight with open-cooked meat was greater than allowed for by the above limits. On the other hand with pressure-cooked meat there was little difficulty in meeting the requirements. Therefore, it was decided to investigate the moisture relations of poultry meat throughout the usual canning operation. Both chicken and fowl were studied and the light and dark meat were treated separately. Moisture content of the meat and solid content of the broth before and after the various heat treatments involved were investigated.

Experimental Procedure

Moisture was determined by cutting a sample of meat into a weighed aluminum drying dish, placing this in an air oven at 100° C. for two hours, and finally drying to constant weight *in vacuo* at 100° C. A period of 16 hr. in the vacuum oven was shown to be sufficient time for samples to reach constant weight and therefore this drying time was used throughout the experiment. The solid content of the broth was determined by drying the sample to constant weight in a small beaker in the air oven at 100° C.

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To investigate sampling technique, the moisture content of various muscles of dressed chickens was determined. The pectoral, leg, and wing muscles were dissected from two birds and their moisture contents were recorded. It was concluded (Table I) that the moisture content of a single muscle or a portion of a muscle, on either side, could be used as an index of the moisture content of the meat of a whole bird.

TABLE I

MOISTURE CONTENT OF VARIOUS MUSCLES OF DRESSED CHICKEN

Chicken	Muscle	Moisture, %	
		Left	Right
A	Outer pectoral	74.0	74.3
	Inner pectoral	73.5	73 9
	Wing	74.5	74.5
	Leg	74.4	74.1
B	Outer pectoral	72.8	72 7
	Inner pectoral	72.8	72.6
	Wing	73.3	73.4
	Leg	71.4	71.0

In the main experiment, three open and three pressure cooks, using four birds in each, were carried out on both chicken and fowl, which were cooked separately. The open cooks were made in a steam-jacketed kettle with the steam outlet from the central chamber open, giving an internal temperature of 100° C. (212° F.). In these cooks approximately one litre of water was used for each 5 lb. of bird. The times were 50, 75, and 100 min. The pressure cooks were done in the same kettle for 20, 30, and 40 min. at 121° C. (250° F., 15 lb. steam pressure). The times were chosen in each case to give under-, normal-, and overcooked meat. Before cooking, all birds were tagged and a portion of the outer pectoral muscle was removed to determine the moisture content of the light meat and a portion of either the gastrocnemius or biceps femoris muscle was taken for the moisture determination of the dark meat. After cooking, portions of the corresponding muscles on the opposite side of each bird were removed and their moisture contents determined.

After sampling, the cooked meat was stripped from the bones and $3\frac{1}{2}$-oz. portions were weighed into 7-oz. cans. Light and dark meat were canned separately. The broth from each cook was defatted, adjusted to a sp. gr. of 1.000 and 1.010 at 50° C. (122° F.) for open- and pressure-cooked meats, respectively, and 2.0% Irish moss gelose was added as a jelling agent along with 4% salt according to the regulations for the packing, grading, and marking of canned poultry (1). When broth samples had been taken for solids determinations, $3\frac{1}{2}$-oz. portions of broth were poured over the meat and the cans were preheated and sealed. All cans were retorted for one hour at 116° C. (240° F.).

Two types of separation apparatus were available, the type described by Reedman (2) and a modification of this apparatus (Fig. 1) designed by Mr. T. A. Steeves, formerly of these laboratories. The rate of heat penetration
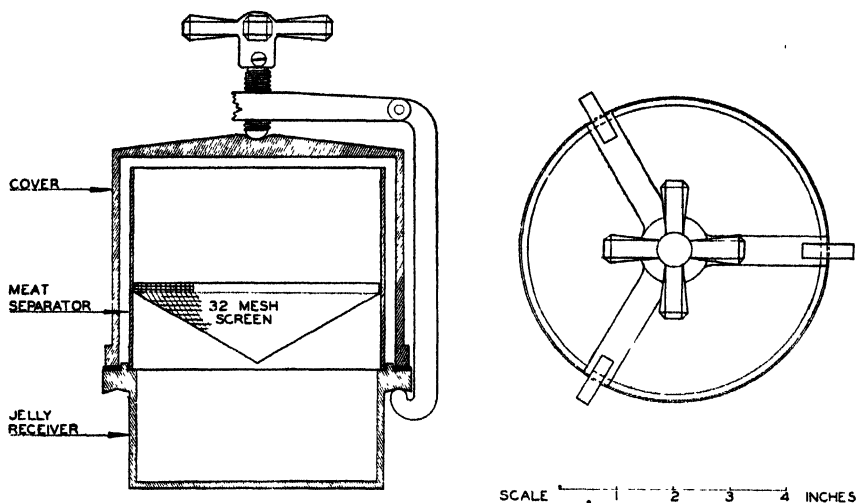
FIG. 1. *Modified separation apparatus for canned poultry.*

into the apparatus was determined using copper–constantan thermocouples. A period of 30 to 40 min. in the autoclave was necessary to raise the meat and jelly inside the separator to 100° C. Accordingly, all separations were made at 121° C. for 45 min. To compare the efficacy of the two types of apparatus, duplicate sets of canned chicken were run in each type.

Since some of the loss of moisture was attributed to the method of separating the meat and jelly, an attempt was made to determine the actual moisture loss due to the separation procedure. However, since it was not possible to separate the meat and jelly completely by means other than heat, no conclusive results were obtained.

Since it is commercial practice in open cooking to cover the birds completely with water and to do several cooks in the same broth, the experiment was repeated in this way. All determinations were carried out as before and, in order to have a valid check, pressure cooks were carried out on birds obtained from the same source. In the open cooks it was necessary to add eight litres of water to cover four birds completely. This was brought back up to volume after each cook. Agar (1%) was used as the gelling agent in this test.

All results were subjected to statistical analysis.

Results

Moisture Changes in Meat

Average moisture losses from chicken and fowl during cooking and the canning and separation procedures are shown in Table II. Necessary differ-

TABLE II

Loss of Original Moisture from Poultry Meat during Cooking and Canning

Treatment	Average percentage loss during cooking	Average percentage loss due to cooking, and to canning and separating	Average percentage loss due to canning and separating
Chicken			
Open cook			
50 min.	13.9*	16.5	2.6
75 min.	17.4	17.4	0.0
100 min.	14.1	16.6	2.5
Pressure cook			
20 min.	19.9	21.6	1.7
30 min	18.8	18.9	0.1
40 min.	21.6	21.4	−0.2
Necessary difference (5%)	2.5	1.8	3.1
Fowl			
Open cook			
50 min.	12.1**	20.0	7.9
75 min.	13.8	18.3	4 5
100 min.	14.4	17.8	3.4
Pressure cook			
20 min.	18.7	19.0	0.3
30 min.	19 8	19.2	−0.6
40 min.	18 8	17.7	−1.1
Necessary difference (5%)	1.4	2.6	2.9

* *Means of 16 observations.*
** *Means of 8 observations.*

ences for a 5% level of significance are also given.   Moisture losses are calculated throughout on the basis of the original weight of raw meat.

These results show that pressure cooking removes a significantly greater amount of the original moisture from both chicken and fowl meat than does open cooking.   The only significant effect of time of cooking noted was with the short time open cook on fowl.   Here the loss of moisture was significantly less than for either of the longer cooks.

The data obtained with chicken meat after separation show the same general effects as those obtained after cooking; the total moisture loss was significantly greater from the pressure-cooked than from the open-cooked meat.   However, the loss due to canning and separating was greater for the open than for pressure-cooked meat, but time of cooking had little effect. Canning and separation after pressure cooking caused the greatest loss in meat that was cooked for 20 min. only.   With the fowl meat, canning and separation eliminated any difference in moisture due to method of cooking since the moisture loss attributable to canning and separation was much greater in the

open- than the pressure-cooked meat and in each instance was inversely proportional to the time of cooking. There are indications that after canning, over-cooked meat may reabsorb some moisture.

The percentage losses of original moisture from both light and dark meat are compared in Table III. It is evident that dark meat lost significantly

TABLE III

PERCENTAGE OF ORIGINAL MOISTURE LOST FROM LIGHT AND DARK CHICKEN MEAT DURING COOKING AND PROCESSING

(Means of 12 observations)

Treatment	Type of meat	Percentage loss of original moisture during cooking	Percentage loss of original moisture after separation	
			Old separator	New separator
Open cook	Light	11.2	15.7	13.5
	Dark	19.1	19.8	18.4
Pressure cook	Light	18.5	19.9	18.2
	Dark	21.7	22.6	21.8
Necessary difference (5%)		2.5	1.4	1.4

more moisture than light meat, the difference being greater in open than in pressure cooking. These differences persisted even after processing and separation. Although the results are not presented, similar differences were noted with fowl meat.

Comparison of the results obtained with the two types of separation apparatus show that the older type dried out the meat considerably more than did the newer one.

TABLE IV

WEIGHT OF MEAT OBTAINED AFTER SEPARATION
(ORIGINAL WEIGHTS, 3.50 OZ.)

Treatment	Chicken, oz.	Fowl, oz.
Open cook		
50 min.	3.05*	2 67**
75 min.	3.06	2.88
100 min.	3.16	3.04
Pressure cook		
20 min.	3.27	3.10
30 min.	3.44	3.37
40 min.	3.46	3.29
Necessary difference (5%)	0.17	0.17

   * Means of 16 observations.
  ** Means of 8 observations.

The average weights of meat obtained after separation are contained in Table IV. These results confirm those obtained in the moisture studies. It will be noted that method has a much greater effect than time of cooking on the weight of meat obtained. In addition, the loss from fowl meat was greater than that from chicken meat for all cooking conditions. There were no significant effects of cooking time with the open-cooked chicken. With pressure-cooked chicken the loss from meat cooked 20 min. was greater than from meat cooked for the longer times. This difference just reaches the 5% level of significance. There was a significantly greater loss of weight from the fowl cooked 20 min. than from fowl that received the longer cooks and that had been dehydrated to a greater extent during the cooking process. It should also be mentioned that there was a significant difference between the weights of light and dark meat obtained after separation from fowl but not from chicken.

As the changes in percentage of solid material in the broth after canning and separating were relatively small and there were no significant differences between cooks, these results have not been included. However, it should be

TABLE V

Loss in Weight (oz.) from Canned Chicken and Fowl During Canning and Separating, together with Estimates of the Weight of Cooked Meat Needed to Obtain 3½ oz. After Canning and Separating

Treatment	Average moisture loss	Average solids loss	Combined loss	Total observed loss	Calculated weight to obtain 3 5 oz. after canning and separation
Chicken					
Open cook					
50 min.	0.34	0.07	0.41	0.45*	4.0
75 min.	0.26	0.08	0.34	0.44	4.0
100 min.	0.28	0 03	0.31	0.34	3.9
Pressure cook					
20 min.	0.18	0.07	0 25	0.23	3 7
30 min.	0.04	0.05	0.09	0.06	3 6
40 min.	0.02	0.05	0.07	0.04	3.5
Fowl					
Open cook					
50 min.	0.71	0.14	0.85	0.83**	4.6
75 min.	0.48	0.14	0.62	0 62	4 3
100 min.	0.36	0.13	0.49	0.46	4 0
Pressure cook					
20 min.	0.27	0.13	0.40	0.40	4.0
30 min.	0.12	0.01	0.13	0.13	3 6
40 min.	0.16	0.06	0.22	0.21	3.6

* *Means of 16 observations.*
** *Means of 8 observations.*

noted that there was a greater, but not significant, loss of solid material to the broth from dark than from light meat.

The entire experiment is summarized in Table V. In this the average losses of both moisture and solids are presented together with the total observed loss in weight. In calculating the loss of solid material to the broth it was assumed that the dry weight lost by the meat in the canning and separation processes was gained by the broth. The principal loss of weight was due to moisture and not to solid material. The contrast between the results for chicken and those for fowl should be especially noted. For every cooking condition used the meat obtained from fowl showed a greater decrease in weight on subsequent processing than did that obtained from chicken. It can be seen from these results that if a can of chicken that has been open cooked is to contain 50% by weight of meat after separation, it must contain about 60% of meat initially. The final column of Table V gives an estimate of the weights of precooked meat that must be canned if $3\frac{1}{2}$ oz. are to be obtained after autoclaving and separation.

Acknowledgments

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FLAVOUR REVERSION IN HYDROGENATED LINSEED OIL

III. THE RELATION OF ISO-LINOLEIC ACID TO FLAVOUR DETERIORATION

By H. W. Lemon

FLAVOUR REVERSION IN HYDROGENATED LINSEED OIL

III.   THE RELATION OF ISO-LINOLEIC ACID TO FLAVOUR DETERIORATION[1]

By H. W. Lemon[2]

Abstract

Further evidence that iso-linoleic acid is related to the unpleasant odour development when partially hydrogenated linseed oil is heated is as follows:

(a) Partially hydrogenated perilla oil, containing considerable iso-linoleic acid, developed the same odour when heated, as did partially hydrogenated linseed oil.

(b) A concentrate of iso-linoleic acid developed a similar odour when it was heated. Distillation of the acid, or removal of unsaponifiable substances, had no effect on the odour development.

(c) When fatty acid fractions obtained by crystallization of the acids from hydrogenated linseed oil were re-esterified with glycerol, and subjected to the heat test, most odour development occurred in the fraction containing most iso-linoleic acid.

Increasing the selectivity of hydrogenation did not greatly affect the formation or hydrogenation of iso-linoleic acid. However, products of high temperature (200° to 250° C.) hydrogenations were softer for equivalent iodine numbers, and had lower melting points than those from low temperature hydrogenations.

Hydrogenation of partly polymerized linseed oil yielded a product that when heated did not develop the characteristic odour of hydrogenated linseed oil.

It was reported in a previous publication (8) that when linseed oil is hydrogenated an isomeric linoleic acid is formed in which the double bonds are in such positions that they will not form a conjugated system upon alkali isomerization. Some experimental evidence was presented that indicated that the isomeric acid may be responsible for the characteristic odour that develops when partially hydrogenated linseed oil is heated to baking and frying temperatures. It was suggested that hydrogenation of linseed oil should be highly selective in order that the iso-linoleic acid may be completely hydrogenated without the formation of large quantities of saturated acids.

In the present paper further evidence that iso-linoleic acid plays a part in flavour development on heating is presented, together with the results of experiments to reduce its amount in the product: (a) by increasing the selectivity of hydrogenation and (b) by reducing the linolenic acid content of linseed oil by polymerization.

Methods

Analytical methods and the techniques used for refining, bleaching, hydrogenation, and deodorizing have been described (8). Linolenic and linoleic acids were determined by the spectral method of Mitchell, Kraybill, and

Zscheile (10) as modified by Brice, Swain, Schaeffer, and Ault (6). Saturated and iso-oleic acids were determined by the method of Baughman and Jamieson (4).

Polymerization of linseed oil was done in a 2 litre three-necked flask equipped with thermometer, a tube for bubbling carbon dioxide through the oil, and a goose neck leading to a condenser. The reaction was carried on under vacuum at 315° C.

Experimental and Results

A. RELATION OF ISO-LINOLEIC ACID TO ODOUR DEVELOPMENT

Hydrogenation of Perilla and Tung Oils

The amount of linolenic acid in perilla oil is as much as, or more than, that in linseed oil, and so perilla oil might be expected to yield a hydrogenation product similar in respect to flavour reversion characteristics to that produced by linseed oil. Tung oil contains a large proportion of eleostearic acid, an isomer of linolenic acid, and it is unlikely that its hydrogenation would produce the same iso-linoleic acid as hydrogenation of linolenic acid. Therefore, a quantity of each of these oils was hydrogenated to a plastic consistency, and samples subjected to the heat test.

The perilla oil was alkali-refined, hydrogenated, and deodorized in the usual manner. Samples were taken for fatty acid analysis, and the results are given in Fig. 1. As in the case of linseed oil, iso-linoleic acid was produced
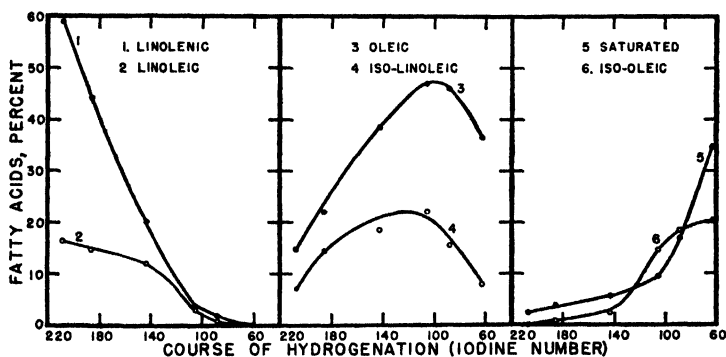
FIG. 1. *Progressive change in fatty acid composition on hydrogenation of perilla oil.*

on hydrogenation. The tung oil was hydrogenated without refining. Samples were examined for diene and triene conjugation before and after alkali isomerization with the use of a Beckman spectrophotometer; the absorption curves are shown in Fig. 2. Saturated and iso-oleic acids were not determined. There was a decrease in triene conjugation on hydrogenation but very little development of diene conjugation. Alkali isomerization had little effect on the amount of diene and triene conjugation present. These results indicate that, when eleostearic acid is hydrogenated under the conditions described, two or more double bonds are saturated simultaneously.

The hydrogenated perilla, linseed, and tung oils, and a standard all-hydrogenated shortening were heated to 200° C. The hydrogenated perilla and hydrogenated linseed oils developed the same unpleasant odour, the two being indistinguishable. The hydrogenated tung oil, with an apparent iodine
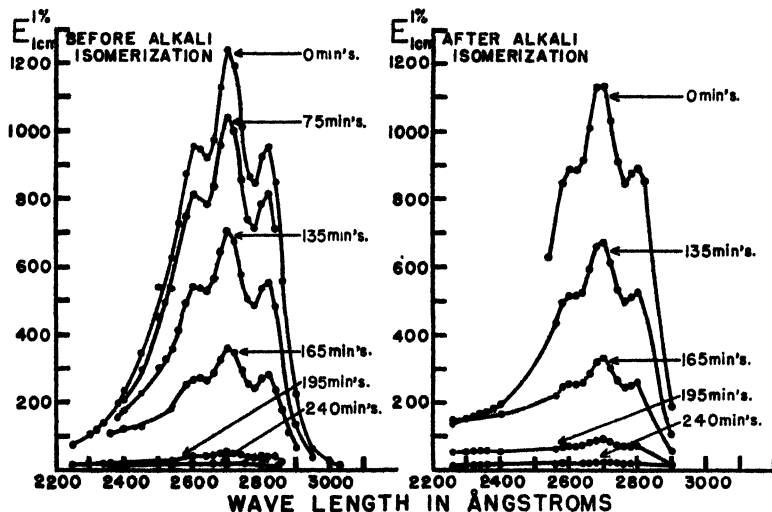
FIG. 2. *Effect of hydrogenation time on ultraviolet absorption by tung oil.*

number of 58.6, did not develop such an odour. This experiment provided, further evidence that the substance responsible for the unpleasant odour of hydrogenated linseed oil is formed by the hydrogenation of linolenic acid.

Heat Tests on Iso-linoleic Acid

It was stated in the first paper of this series (8) that a strong odour resembling that of heated hydrogenated linseed oil developed on heating a distilled iso-linoleic acid concentrate. The odour was quite different from that of linolenic, linoleic, or oleic acids treated in the same way.

It was thought that the odour might be caused by some unsaponifiable substance associated with the iso-linoleic acid. Therefore a quantity of the acid in alcoholic solution was neutralized with potassium hydroxide, diluted with water, and the soap solution extracted several times with ether. The acids were liberated from the soap, washed with water, dried, and distilled from a Hickman alembic flask. On heating, the acids developed the same strong odour, resembling that of hydrogenated linseed oil.

Fractional Crystallization of Fatty Acids from Hydrogenated Linseed Oil

The isolation of a concentrate of iso-linoleic acid by low temperature crystallization of the separated fatty acids of hydrogenated linseed oil was described in the first paper of this series (8). In a similar separation, four fatty acid fractions were obtained. The first of these consisted of the acids that precipitated from acetone at −30° C.; the second fraction precipitated

at from −30° to −60° C. The third fraction consisted of all of the crystalline precipitates that could be obtained by chilling to −60° C. the concentrated filtrate from the previous precipitations, and the fourth fraction, consisting largely of iso-linoleic acid, was obtained from the final filtrate.

Each of these fractions was esterified with glycerol in the proper amount to yield triglyceride. The products were deodorized. The fat made from the first fraction was quite hard, that from the second fraction was semisolid, and those from the third and fourth fractions were liquid at room temperature.

The four fats were heated to 200° C. There was little, if any, development of the reverted odour in the first fat but the fourth fat developed a very strong odour. Numbers two and three were similar, and intermediate to numbers one and four. There was no doubt that the odour development was caused by heating the iso-linoleic glyceride itself, or some substance associated with it.

B. INFLUENCE OF SELECTIVITY ON HYDROGENATION OF ISO-LINOLEIC ACID

It was felt that hydrogenated linseed oil would be more suitable for use as a shortening if the iso-linoleic acid content were reduced or eliminated, and it was thought that some improvement might be made by increasing the selectivity of hydrogenation. It has been pointed out by Bailey, Feuge, and Smith (2) that selectivity is increased by raising the temperature or catalyst concentration, and by decreasing the pressure or agitation, and that each of the conditions contributing to selectivity causes a greater formation of iso-oleic acid.

Three series of hydrogenations were completed. In the first series the temperature was kept low during the initial period of the hydrogenations but was raised rapidly when the refractive index (Zeiss butyro) had dropped to about 58 at 40° C. corresponding to an iodine number of approximately 120; at the same time the gauge pressure was reduced from 25 to 5 lb. It was hoped that increasing the temperature at about iodine number 120 would suppress the rapid formation of saturated acids which normally begins at about this point, and at the same time favour the hydrogenation of iso-linoleic acid. Various temperature combinations were tried. In the second and third series of hydrogenations, four temperatures between 120° and 250° C. were selected, and the oil and catalyst mixtures were brought to these temperatures as rapidly as possible. A slight positive pressure of hydrogen was maintained during the period required to bring the oil to the desired temperature; it was then increased to 25 lb. gauge pressure.

In all three series, samples were taken at intervals during the hydrogenations for fatty acid analysis. The results are given in Table I. By plotting these results against iodine number, saturated and iso-linoleic acid values at iodine number 90 were determined for the low to high temperature series. These values are given in Table II. It is apparent that raising the temperature of hydrogenation at iodine number 120 had no appreciable effect on the subsequent hydrogenation of iso-linoleic acid.

TABLE I

ANALYSIS OF HYDROGENATED LINSEED OIL SAMPLES

Hydrogena-tion temperature, °C.	Time, min.	Iodine number	Fatty acid composition, %					
			Saturated	Iso-oleic	Oleic	Iso-linoleic	Linoleic	Linolenic

Series I.   Low to high temperature hydrogenations

140	20	148.5	7.4	1.9	40.8	5.6	15.2	29.1
140	40	120.8	10.1	4.0	48.1	14.9	10.9	12.0
140	60	94.4	17.0	7.5	50.9	17.1	5.4	2.1
140	70	79.6	23.1	10.7	50.1	13.8	1.8	0.5
115-190	30	163.2	6.5	0.9	36.0	1.5	17.0	38.1
115-190	75	119.6	10.9	3.4	46.9	16.8	11.5	10.5
115-190	100	97.8	13.6	9.0	51.7	20.5	3.9	1.3
115-190	115	79.2	21.5	19.0	45.9	13.5	0.1	0
115-240	45	147.0	7.4	1.5	40.1	8.2	16.6	26.2
115-240	80	112.4	11.9	3.9	48.6	18.7	10.2	6.7
115-240	95	89.0	15.0	15.4	51.4	16.6	1.2	0.4
115-240	100	82.8	17.1	22.8	46.6	13.4	0.1	0
140-190	20	152.3	8.2	2.3	36.3	5.1	17.1	31.0
140-190	50	108.1	12.2	6.9	48.3	19.4	8.2	5.0
140-190	60	96.4	14.3	12.1	49.0	18.9	4.1	1.6
140-190	75	81.9	17.7	16.7	52.7	12.7	0.2	0
140-235	25	146.0	7.9	2.1	39.6	7.8	16.4	26.2
140-235	45	106.7	12.1	5.5	51.6	17.4	8.2	5.2
140-235	55	84.3	18.2	16.4	50.3	12.9	1.2	1.0
140-235	60	72.0	23.0	28.8	41.0	6.4	0.5	0

Series II.   Effect of temperature of hydrogenation on selectivity

120	60	132.5	10.0	2.8	40.3	18.3	12.4	16.2
120	90	106.4	14.6	6.0	46.4	20.4	7.7	4.9
120	120	88.1	20.9	8.3	49.0	16.6	3.8	1.4
120	155	70.5	31.2	10.0	46.0	11.6	1.0	0
150	30	128.0	10.3	3.3	42.1	18.2	12.2	13.9
150	45	103.7	15.2	6.3	47.3	20.4	6.5	4.3
150	60	80.3	25.3	9.7	48.8	15.2	1.0	0.8
150	80	53.9	43.7	10.8	39.2	6.1	0.2	0
200	25	93.5	14.6	14.3	49.3	18.1	2.3	1.4
200	30	80.1	20.4	15.9	50.7	12.0	0.6	0.4
200	40	59.9	36.4	17.9	39.7	5.7	0.3	0
200	45	47.8	47.7	14.3	34.7	3.2	0.1	0
250	20	95.6	12.0	17.6	48.7	17.5	2.9	1.3
250	25	80.9	18.8	23.4	45.0	12.2	0.6	0
250	30	67.5	26.8	27.6	40.3	5.1	0.2	0
250	35	56.8	37.6	20.4	38.3	3.6	0.1	0

TABLE I—*Continued*

ANALYSIS OF HYDROGENATED LINSEED OIL SAMPLES—*Continued*

Hydrogena-tion temperature, °C.	Time, min.	Iodine number	Fatty acid composition, %					
			Saturated	Iso-oleic	Oleic	Iso-linoleic	Linoleic	Linolenic

Series III. Effect of temperature of hydrogenation on selectivity

120	60	146.0	9.7	2.5	32.6	18.1	13.8	23.3
120	120	105.6	16.4	8.5	40.2	21.4	6.5	6.0
120	160	80.4	27.2	11.3	42.0	16.1	2.3	1.1
120	170	72.8	30.9	10.3	44.0	12.9	1.1	0.8
120	190	58.7	39.7	9.5	42.7	7.5	0.6	0
150	25	135.4	9.3	4.3	38.9	17.0	12.2	18.3
150	50	103.7	12.6	12.2	46.6	18.9	5.4	4.3
150	65	83.9	19.7	17.0	46.0	16.2	1.1	0
150	75	75.0	25.7	17 3	42.1	13.4	0.5	0
150	95	49.8	45.9	16.5	33.6	4.0	0	0
200	25	116.6	8.6	12.0	45.4	15.4	9.0	9.6
200	33	93.8	10.9	26.0	43.7	18.1	0.8	0.5
200	42	82.2	16.5	32.9	39.5	11.1	0	0
200	51	67.8	27.1	30.7	36.2	6.0	0	0
200	55	62.0	31.8	27.9	36.1	4.2	0	0
240	20	115.8	9.6	11.8	43.2	18.7	8.4	8.3
240	24	103.1	10.3	18.4	43.6	21.1	4.4	2.2
240	33	90.7	13.6	22.6	44 8	17.8	1.2	0
240	45	78.0	19.3	23 4	47.4	9.5	0.35	0
240	65	65.7	27.8	23.6	43.3	5.3	0	0

TABLE II

EFFECT OF RAISING THE TEMPERATURE DURING HYDROGENATION ON
THE CONTENT OF SATURATED AND ISO-LINOLEIC
ACIDS AT IODINE NUMBER 90

Hydrogenation temperature, °C.	Saturated acids, %	Iso-linoleic acid, %
140	19.0	16.5
115-190	16.5	18.5
115-240	15.0	16.5
140-190	15.5	17.5
140-235	16.0	15.0

In Fig. 3 the effect of temperature of hydrogenation on fatty acid composition at iodine numbers 80 and 90 is shown for Series II hydrogenations. Increasing the temperature caused more complete hydrogenation of linolenic and linoleic acids, increased formation of iso-oleic acid, and decreased produc-

tion of saturated acids; however, it had little effect on the hydrogenation of iso-linoleic and oleic acids. These conclusions are in agreement with those of Bailey and Fisher (3), who found that iso-linoleic acid is much more resistant to hydrogenation than linoleic or linolenic acids.
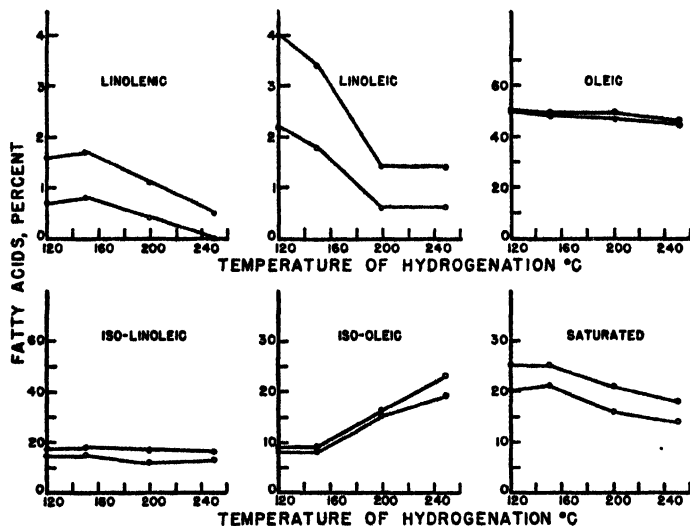
FIG. 3. Effect of temperature of hydrogenation on the fatty acid composition of hydrogenated linseed oil.

The melting points of samples from Series III hydrogenations were determined by the capillary tube method, and their micropenetration values at 25° C. were determined with a micropenetrometer similar to that described by Feuge and Bailey (7). The values obtained have been plotted against iodine numbers (Fig. 4). It is evident that the melting points of samples taken from the high temperature hydrogenations were lower for equivalent iodine numbers than those of samples taken from the low temperature hydrogenations. Similarly, the penetration values at 25° C. of samples from the high temperature hydrogenations were greater than those of samples of the low temperature hydrogenations for equivalent iodine numbers, although the difference became less as hydrogenation proceeded.

From the results, the iodine number corresponding to a melting point of 47° C. has been estimated for hydrogenations done at 250°, 200°, 150°, and 120° C., respectively, and the fatty acid composition for each of these iodine numbers has been determined from the results in Table I. These values are given in Table III.

It can be concluded that if linseed oil is hydrogenated at a high temperature, it can be saturated to a lower iodine number without becoming too hard, giving a product with a lower iso-linoleic acid content. However, when samples having a lower iso-linoleic acid content were heated, there was still a

strong odour development, and it was difficult to determine whether there was any improvement.
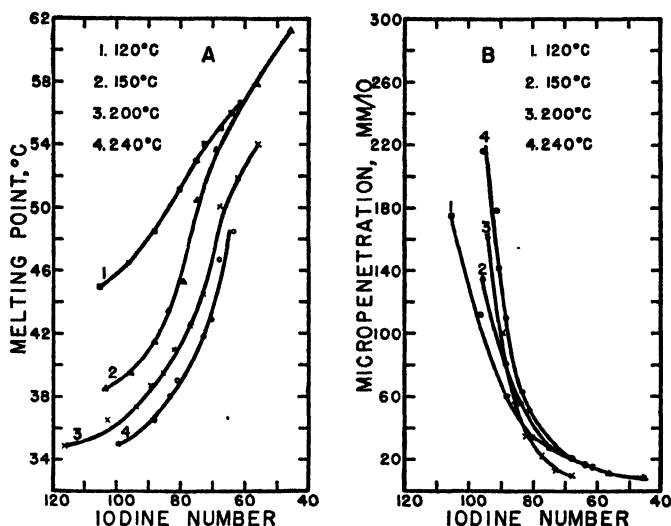
FIG. 4. *Effect of temperature of hydrogenation of linseed oil on melting point (Graph A) and on micropenetration at 25° C. (Graph B).*

TABLE III

EFFECT OF TEMPERATURE OF HYDROGENATION OF LINSEED OIL ON THE FATTY
ACID COMPOSITION AT MELTING POINT OF 47° C.

Hydrogenation temperature, °C.	Iodine number	Fatty acid composition, %					
		Saturated	Iso-oleic	Oleic	Iso-linoleic	Linoleic	Linolenic
120	94	21.2	10.3	41.7	19.2	4.4	3.3
150	79	22.5	17.2	44.5	14.5	0.7	0
200	70	25.0	31.4	37.0	6.5	0	0
240	66	27.5	23.6	43.5	5.5	0	0

C. HYDROGENATION OF POLYMERIZED LINSEED OIL

When linseed oil is polymerized, there is a reduction in the linolenic and linoleic acids content.   There is a commercial process in operation that entails polymerization followed by extraction of the non-polymerized portion with acetone (5).   As this results in an oil with a low concentration of linolenic acid it has been suggested that the oil might be used as an edible oil.   Privett, Pringle, and McFarlane (11) have developed a similar process, involving milder polymerization, for the purpose of obtaining an edible fraction from linseed oil.   In view of this work it was decided to determine quantitatively by the spectral method the disappearance of linolenic and linoleic acids on

polymerization, and to hydrogenate oils polymerized to varying degrees and apply heating tests in order to determine their susceptibility to reversion.

Polymerization was carried on for 20 min., one hour, two hours, and three hours. Refractive index, free fatty acid, and linolenic and linoleic acids were determined for each product. The results are given in Table IV. It is evident that, although the disappearance of linolenic acid on polymerization is rapid, there is still some present after heating for three hours.

TABLE IV

POLYMERIZATION OF LINSEED OIL

Time of polymerization	Refractive index, 50° C.	Free fatty acid, % as oleic	Linoleic acid, %	Linolenic acid, %
0 min	1.4699	0.05	19.6	51.9
20 min.	1.4715	1.2	14.4	43.1
1 hr.	1.4744	1.8	13.9	26.6
2 hr.	1.4771	2.8	11.2	13.9
3 hr.	1.4790	3.7	9.1	7.5

Each of the polymerized oils was hydrogenated without any further treatment. The hydrogenations proceeded with some difficulty, probably owing to the high free fatty acid content. The products were deodorized, and samples heated to 200° C. on a hot plate. The hydrogenated linseed odour was present in the product from the 20 min. polymerization, but there was little, if any, of it in the others.

Discussion

There is considerable evidence that the unpleasant flavour and odour of heated hydrogenated linseed oil are caused by the decomposition of iso-linoleic acid, although there is some indication from storage tests (9) that this is not the only cause of flavour deterioration. Reversion in unhydrogenated oils, such as soybean, is not explained by the iso-linoleic acid theory, and it is probable, as has been suggested by Bailey (1), that in this case it is the result of a different mechanism.

If iso-linoleic acid plays a part in reversion its removal from hydrogenated linseed oil presents a difficult problem. The results of the experiments to determine the effect of selectivity on its hydrogenation were not encouraging, as it apparently hydrogenates with difficulty, and quantities of it persist until the product has become very hard regardless of the conditions of hydrogenation.

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RATION BISCUITS

IV. EFFECT OF TEMPERATURE AND SHORTENING TYPE ON KEEPING QUALITY[1]

By H. J. Lips[2]*, N. C. Crowson[3], and W. Harold White[4]

Abstract

Biscuits commercially prepared with shortenings of four types (compound animal–vegetable, blended vegetable, hydrogenated vegetable, and stabilized, hydrogenated vegetable) were coarsely ground and stored in sealed cans at 26.7°, 43.3°, and 60° C. (80°, 110°, and 140° F.). Deterioration was assessed by peroxide oxygen and pH determinations, and by flavour scores. The results obtained for biscuits stored at 60° C. were not indicative of behaviour at the lower temperatures. Flavour score was the most useful estimate of biscuit keeping quality, especially at 26 7° and 43 3° C. Peroxide development was appreciable only in biscuit material stored at 26.7° C.

Biscuits prepared with hydrogenated vegetable shortenings were generally more stable (average storage life: 84, 22, and 13 weeks at 26.7°, 43.3°, and 60° C., respectively) than those prepared with animal–vegetable or blended vegetable shortenings. However, shortenings with Swift stabilities (110° C.) greater than 40 hr. did not yield products of increased keeping quality

Introduction

One of the important causes of spoilage in baked goods is deterioration of the fat used in their preparation. Therefore it might be expected that the keeping quality of a baked product would bear a direct relation to the keeping quality of the shortening used. However, determination of shortening stability does not necessarily indicate the stability that the shortening will show in soda crackers (4, 12, 13). This has been attributed in part to destruction of antioxidants and peroxides in the fat during baking.

A previous investigation in these laboratories demonstrated that ration biscuits had a longer storage life at 43.3° C. when a stabilized, hydrogenated vegetable, rather than a compound, animal–vegetable shortening was used (5). The present paper describes an examination of a number of biscuit shortenings and the results obtained from storage tests on ration biscuits prepared with these shortenings.

Materials and Methods

Seventeen currently available shortenings (1943) were classified according to information received from the manufacturers into four general groups: I,

compound animal–vegetable; II, blended all vegetable; III, hydrogenated vegetable; and IV, stabilized, hydrogenated vegetable. Keeping quality was estimated by Swift stability tests at 110° C. (9) and by peroxide measurements (15) at 10 appropriate intervals on samples stored in loosely covered half-pint jars for 38 weeks at 26.7° C. Capillary melting point (15), Kaufman iodine number '(15), refractive index at 48° C. (15), saponification number (1), and smoke point (1) were determined on the fresh material.

The experimental biscuits were prepared by two commercial manufacturers. Processing was carried out according to the formula: 50 lb. of soft wheat flour, 5 lb. of shortening, baking soda (6 oz., Plant A; 8 oz., Plant B), and water as used in plant practice. Shortenings (described above) and flour used by both manufacturers were obtained from the same sources. The amount of moisture in the biscuits produced was quite uniform, but the fat content was generally higher for Plant A products (Table I).

TABLE I

MEAN MOISTURE AND FAT CONTENTS OF BISCUITS PREPARED WITH FOUR
TYPES OF SHORTENING IN TWO PLANTS

Biscuit group	No. of samples in group		Fat and moisture contents, %			
			Plant A		Plant B	
	Plant A	Plant B	Fat	Moisture	Fat	Moisture
I. Compound animal–vegetable shortenings	4	3	10.4	7.5	7.8	7.1
II. Blended vegetable shortenings	2	2	10.7	6.6	8.4	6.5
III. Hydrogenated vegetable shortenings	6	3	10.2	7.3	8.0	6.6
IV. Stabilized, hydrogenated vegetable shortenings	5	4	10.3	7.3	8.0	6.8

Each lot of biscuits was sampled and ground. Portions of the material (120 gm.) were placed in laminated glassine bags, and each bag was sealed in a No. 1 tin can. This left about one inch of headspace.

Biscuits prepared by Plant A with 12 of the 17 shortenings were stored at 26.7°, 43.3°, and 60° C., while those prepared with the five remaining shortenings were stored at 60° C. Biscuits made with the same 12 shortenings at Plant B were stored only at 60° C. The plant products were compared at 60° C. because this is the usual temperature employed in the Schaal incubation test for fats and fat-containing materials (6, p. 125). Flavour score of the biscuit, peroxide oxygen value of the extracted fat, and pH of a potassium chloride extract of the biscuit material were determined at each sampling (5).

Samples were taken at 15 suitable intervals during total storage periods of 82, 48, and 30 weeks at temperatures of 26.7°, 43.3°, and 60° C., respectively.

Shortening and biscuit data were examined statistically by means of analyses of variance and by calculation of simple coefficients of correlation.

Results

Shortenings

Group mean values for measured properties are shown in Table II, and peroxide development is further illustrated in Fig. 1.

TABLE II

MEAN VALUES FOR MEASURED PROPERTIES OF 17 BISCUIT SHORTENINGS OF FOUR TYPES

Shortening group	No. of samples in group	Measurements						
		Melting point, ° C.	Iodine No.	Refractive index (at 48° C.)	Saponi-fication No.	Smoke point, ° F.	Swift stability, hr. at 110° C.	Mean peroxide value, ml. of 0.002 N thiosulphate per gm. (over all samplings for 38 weeks' storage at 26.7° C.)
I. Compound animal-vegetable	4	45.1	70.5	1.4589	192.7	420	9	126.8
II. Blended vegetable	2	42.5	77.9	1.4591	192.2	426	13	22.9
III. Hydrogenated vegetable	6	43.8	68.1	1.4579	189.9	425	20	11.4†
IV. Stabilized, hydrogenated vegetable	5	43.6	59.0	1.4568	190.8	424	66	0.8‡
Smallest necessary difference between any two groups for statistical significance (5% level)		—	8.9	0.0012	—	—	12	30.9

† *Three shortenings only.*
‡ *Four shortenings only.*

Statistical calculation showed no significant differences in mean value between shortening groups for melting point, saponification number, and smoke point; significant differences for iodine number and refractive index; and highly significant differences for Swift stability and mean peroxide value. Group IV shortenings were lower in iodine value than those of Groups I, II, and III and lower in refractive index than those of Groups I and II. Swift stability was higher for Group IV than for the other three groups and mean peroxide value was higher for Group I than for the other three.

Iodine number, refractive index, Swift stability, and log mean peroxide value were interrelated (Table III). Low iodine number was associated with low refractive index and high stability. Saponification number was inversely related to smoke point.

TABLE III

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN MEASURED PROPERTIES
OF BISCUIT SHORTENINGS

Quantities correlated	Saponifica-tion No.	Iodine No.	Smoke point	Refractive index	Melting point	Swift stability
Iodine No.	.11	—	—	—	—	—
Smoke point	− .52*	− .01	—	—	—	—
Refractive index	.11	.77**	.14	—	—	—
Melting point	− .17	− .09	.12	.11	—	—
Swift stability	− .30	− .63**	.16	− .61**	− .10	—
Log mean peroxide value†	.31	.69*	− .28	.74**	.18	− .82**

† *Twelve values; all other measurements, 17 values.*
* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*
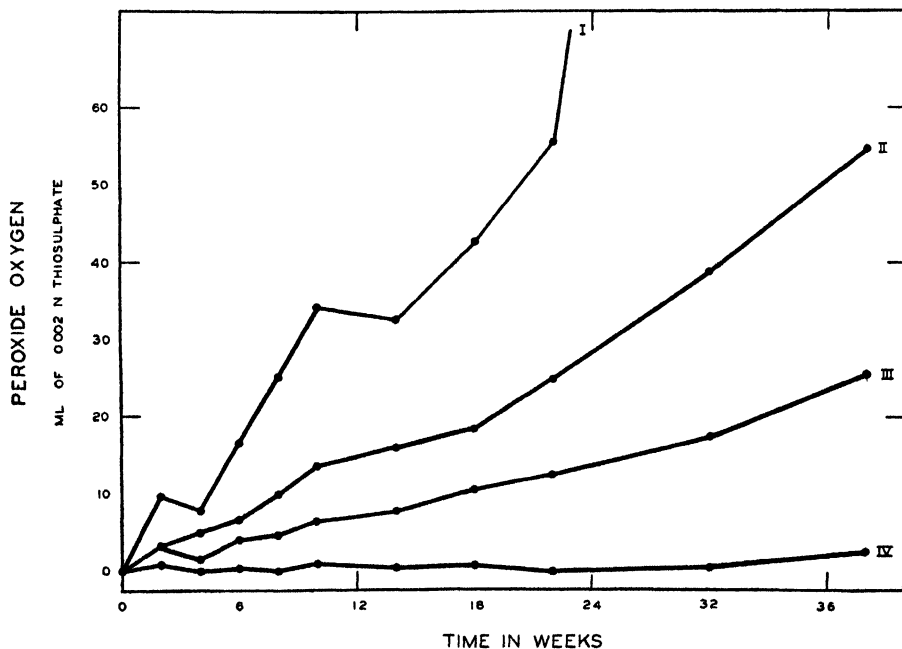
FIG. 1. *Mean peroxide oxygen development in shortenings of four types stored at 26.7° C. for 38 weeks. I, compound animal–vegetable; II, blended all-vegetable; III, hydrogenated vegetable; IV, stabilized, hydrogenated vegetable.*

The results indicate that stability was a function of shortening type. Differences in shortening stability were apparently maintained during storage (Fig. 1). Stabilization of Group IV shortenings was evidently due in part to hydrogenation to a low iodine number, while the inferior keeping quality of Group I material was presumably caused by the presence of animal fat.

Biscuits

Mean values over all sampling periods for flavour, peroxide oxygen, and pH measurements on biscuits made with each of the four types of shortening are presented in Table IV.

There were no statistically significant differences in mean pH and peroxide measurements at any of the storage temperatures and no differences in flavour score of material stored at 60° C. Significant group differences in flavour score were present in biscuits stored at 43.3° C., and highly significant differences in biscuits stored at 26.7° C. The mean flavour score for Group I biscuits was lower than the means of Groups II, III, and IV for storage temperature 26 7° C., and lower than the means of Groups III and IV for storage temperature 43.3° C.

The marked inferiority in keeping quality of Group I biscuits and the lack of differentiation among the other three groups are illustrated by the flavour changes shown in Fig. 2. The effect of storage temperature on mean flavour values averaged over all shortenings is shown in Fig. 3 (Plant *A* biscuits only). It was estimated that ration biscuits prepared with a good grade of hydrogenated vegetable shortening had average storage lives of 84, 22, and 13 weeks at 26.7° C., 43.3° C., and 60° C., respectively.

The chemical measurements yielded only limited information about keeping quality. Pronounced peroxide oxygen development was found only in biscuits prepared with compound animal–vegetable and blended all-vegetable shortenings and stored at 26.7° C. (Fig. 2). There was an initial decline in pH which was much more rapid at the higher storage temperatures (Fig. 3).

Association between mean biscuit flavour and mean peroxide oxygen content of stored shortening was negative, and closer for the lower biscuit storage temperatures (Table V). The relation between peroxide oxygen values for biscuits and for stored shortenings was not significant.

Mean biscuit flavour at 26.7° C. did not increase appreciably with the use of shortenings having Swift stabilities greater than 40 hr. at 110° C. (Fig. 4). A similar relation for Swift stability of shortenings and mean peroxide oxygen content of biscuits held at 26.7° C. is also shown in Fig. 4. Shortening stability and biscuit flavour score were more markedly associated at the lower storage temperatures (Table V). Log Swift stability and mean biscuit peroxide value at 26.7° C. were also related.

Useful storage life of the biscuits was held to be at an end when the flavour score fell to five palatability units (5). Estimated storage lives of the various

TABLE IV

MEAN VALUES OVER ALL SAMPLINGS FOR FLAVOUR SCORES, AND pH AND PEROXIDE OXYGEN VALUES OF RATION BISCUITS STORED AT 26.7°, 43.3°, AND 60° C., FOR 82, 48, AND 30 WEEKS, RESPECTIVELY

Biscuit group	No. of samples in group		Flavour score				pH				Peroxide oxygen value, ml. of 0.002 thiosulphate per gm. of fat			
	Plant A, 60° C.	All others	Plant A			Plant B	Plant A			Plant B	Plant A			Plant B
			26.7° C.	43.3° C.	60° C.	60° C.	26.7° C.	43.3° C.	60° C.	60° C.	26.7° C.	43.3° C.	60° C.	60° C.
I. Compound animal-vegetable shortenings	4	3	4.9	4.6	5.3	5.5	8.1	7.7	7.2	7.6	23.3	7.3	1.5	0.3
II. Blended vegetable shortenings	2	2	6.2	5.1	5.3	5.7	7.9	7.5	7.2	7.5	31.8	6.9	1.4	0.2
III. Hydrogenated vegetable shortenings	6	3	6.1	5.7	5.5	5.8	7.9	7.6	7.2	7.5	10.5	1.7	0.3	0.1
IV. Stabilized, hydrogenated vegetable shortenings	5	4	6.4	5.8	5.4	5.7	8.0	7.6	7.2	7.5	3.3	1.1	0.4	0.1
Smallest necessary difference between any two groups for statistical significance (5% level)			0.8	0.9	—	—	—	—	—	—	—	—	—	—

FIG. 2.   *Changes in mean flavour score and peroxide value for biscuits stored at 26.7° C. and prepared with four shortening types: I, compound animal–vegetable; II, blended all-vegetable; III, hydrogenated vegetable; IV, stabilized, hydrogenated vegetable.*

groups of biscuits under the storage conditions studied are given in Table VI. Calculations based on these data supported the previous conclusions, namely, that a high level of biscuit keeping quality was obtained by the use of a reasonably stable shortening, but further increase in biscuit life was not obtained by the use of shortenings of still greater stability.
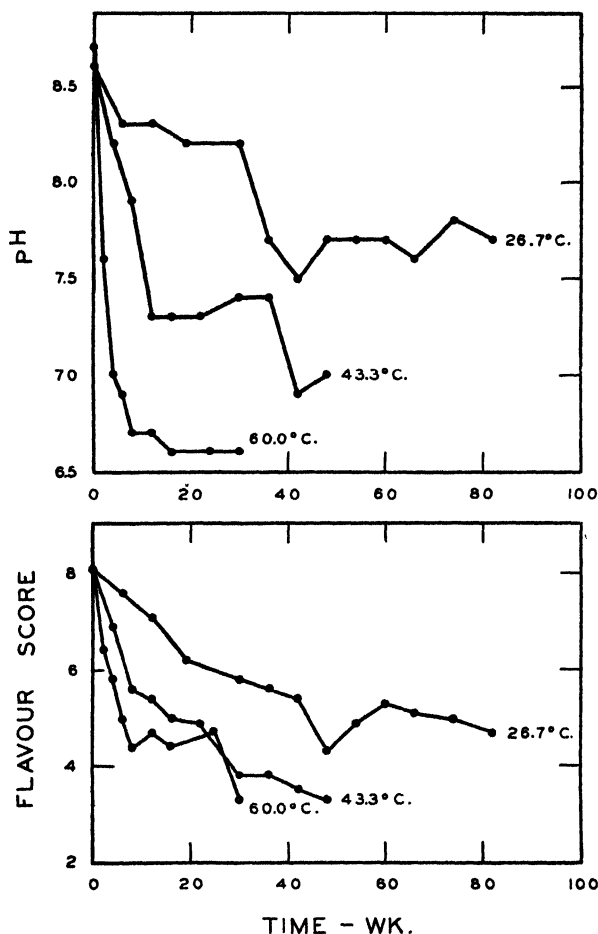
FIG. 3. *Changes in mean flavour score and pH value, over all shortenings used, for biscuits stored at 26.7°, 43.3°, and 60.0° C. (Plant A only).*

No conclusion could be drawn regarding plant practice, as the storage temperature (60° C.) at which the two plant products were compared was the one at which differentiation among biscuit groups was least pronounced. The use of different quantities of baking soda (8, 10) and the variation found in fat concentration (5), together with differences in processing procedure, probably accounted for differences between the plant products.

TABLE V

CORRELATION OF SHORTENING STABILITY WITH BISCUIT STABILITY

Quantities correlated			Correlation coefficient
Mean peroxide values of 12 stored shortenings (26 7° C.) with:			
Plant *A*	Mean biscuit flavour,	26.7° C.	− .92**
		43.3° C.	− 74**
		60° C.	− .55*
	Mean biscuit peroxide value, 26 7° C.		.53
Plant *B*	Mean biscuit flavour,	60° C.	− .56*
Log Swift stability (110° C.) of 12 shortenings with:			
Plant *A*	Mean biscuit flavour,	26.7° C.	.78**
		43.3° C.	.81**
		60° C.	.56*
	Mean biscuit peroxide value, 26.7° C.		− .78**
Plant *B*	Mean biscuit flavour,	60° C.	.21

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

Discussion

Shortenings

Mean iodine numbers of 75.2 for 11 samples of mixed animal–vegetable shortening, 78.2 for 31 samples of blended all-vegetable shortening, and 69.9 for 60 samples of hydrogenated vegetable shortening have been reported (2). These figures are somewhat higher than those reported here.   An examination of smoke points determined by other workers (14) shows a comparable average of 422° F. for 10 vegetable shortenings.

None of the correlations estimated was sufficiently close for prediction purposes, although a number of anticipated associations were found.   An association of melting point with iodine value was not expected in a selection of shortenings differing so widely in composition.   Decrease in smoke point with increase in saponification value may have been due to variation in free fatty acid content (7, vol. 1, p. 394) or variation in content of component acids of low molecular weight (3, p. 68).

Biscuits

The investigation demonstrated that values for chemical measurements and flavour tests obtained for ration biscuits stored at the usual temperature of 60° C. (6, p. 125) may be misleading if they are used to predict storage behaviour at lower temperatures.

An explanation of the low peroxide value of extracted fat at the higher storage temperatures is possible if it is assumed that breakdown of peroxides

proceeded simultaneously with their formation, and that the relative rate of
peroxide decomposition was greater at the higher temperatures. The more
rapid initial decline in pH at the higher temperatures would support this, if
the lowering of pH was entirely due to formation of free fatty acids from
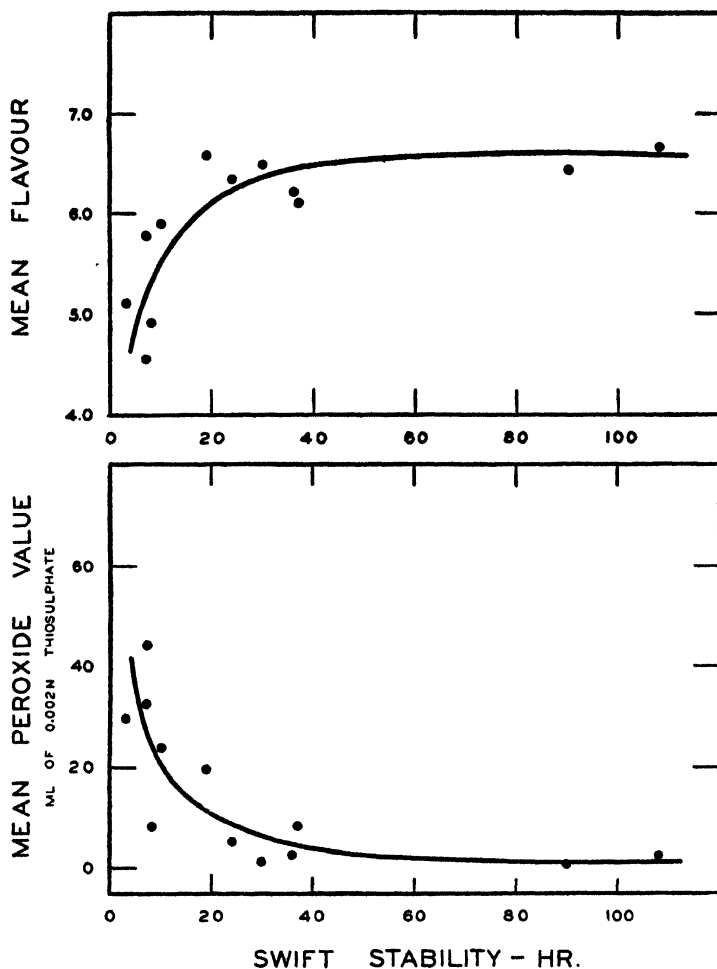
FIG. 4. *Relation of Swift stability (110° C.) of shortenings to mean flavour score and
mean peroxide value of biscuits stored at 26.7° C.*

peroxide breakdown. However, pH changes might be attributable in part to
changes in protein constituents, and it is also possible that decomposition
products of proteins inhibited peroxide formation in the fat fraction,
particularly at 60° and 43.3° C. Baking operations and the influence of
non-fat constituents in the biscuit were apparently responsible for the lack
of close association between peroxide oxygen values of shortenings stored at

TABLE VI

Biscuit group	No. of samples in group		Storage life, weeks			
			Plant A			Plant B
	Plant A, 60° C.	All others	26.7° C.	43.3° C.	60° C.	60° C.
I. Compound animal–vegetable shortenings	4	3	19	7	6	8
II. Blended vegetable shortenings	2	2	63	14	7	13
III. Hydrogenated vegetable shortenings	6	3	67	20	7	17
IV. Stabilized, hydrogenated vegetable shortenings	5	4	97	23	7	17

26.7° C. and peroxide oxygen values of the same fat extracted from biscuits also stored at that temperature. Difference in peroxide development under the two conditions was most marked for the blended all-vegetable shortenings (cf. Figs. 1 and 2).

Although peroxide values for biscuits stored at 26.7° C. and prepared from compound animal–vegetable (Group I) and blended vegetable (Group II) shortenings were both high, biscuits containing the all-vegetable product were rated as having higher mean flavour. This was presumably due to the greater stability of peroxides formed in vegetable fat, as compared to those formed in animal fat, with a corresponding delay in liberation of objectionable products of peroxide breakdown (11).

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FLAVOUR REVERSION IN HYDROGENATED LINSEED OIL

IV. FURTHER PROCESSING STUDIES

By H. J. Lips, H. W. Lemon, and G. A. Grant

FLAVOUR REVERSION IN HYDROGENATED LINSEED OIL

IV. FURTHER PROCESSING STUDIES[1]

By H. J. Lips[2]*, H. W. Lemon[3], and G. A. Grant[4]

Abstract

Flavour reversion could not be detected in samples of hydrogenated linseed oil stored under vacuum in the dark. In samples exposed to the air, reversion occurred without appreciable increase in peroxide oxygen or Kreis values, particularly in products of low iodine number, while accompanying changes in fluorescence were slight and erratic. An observed 'bad area' for susceptibility in the lower iodine number range suggests that iso-linoleic acid (4) is not the only cause of reversion. No improvement in flavour stability was obtained by: low to high temperature hydrogenation (110° to 240° C. (230° to 464° F.)), removal of impurities from the oil, or the use of a linseed oil fraction from a commercial polymerization process.

A previous paper in this series (6) dealt with storage studies to determine the effect of various modifications in processing procedure upon the susceptibility of hydrogenated linseed oil to flavour reversion. The present investigation is an extension of that work.

Experimental

Standard processing techniques, the method of estimating storage life by flavour scores, and some of the chemical measurements used have been described (4, 6). In this study some of the samples were stored at 60° C., rather than at 43.3° C., to obtain comparative results in a shorter time. As earlier work demonstrated that any chemical changes accompanying flavour reversion would be difficult to detect, sensitive modifications of the peroxide oxygen (7) and Kreis (12, 13) tests were introduced as estimates of oxidative deterioration. To investigate possible changes in fluorescence, readings of xylol solutions of the fat were made in a Coleman photofluorometer (3).

Vacuum Storage

It has not been clearly established that the presence of oxygen is essential for flavour reversion (10). To examine the effects of oxygen-free storage on hydrogenated linseed oil, melted samples were evacuated in glass tubes on a high vacuum apparatus for four hours, and sealed off at a pressure of 5μ. These tubes were stored in the dark at 43.3° C. together with an untreated control sample of the same shortening.

The control sample developed definite flavour reversion in 11 weeks while the vacuum-stored material had no detectable reverted odour at the end of 60 weeks' storage. The control had a peroxide value of 2.4 ml. of 0.002 N thiosulphate per gm. (iodimetric method) at the end of 11 weeks. At 60 weeks the peroxide value of the vacuum-stored material was nil, even by the more sensitive ferrometric procedure.

Removal of Impurities

A number of attempts have been made to account for flavour reversion on the basis of impurities in oils. Voskresenkii and Dobruinina (11), working on soybean oil, reported an odorous constituent that had a variable nitrogen content and reduced Fehling's solution. Marcelet (8) isolated from vegetable oils saturated and unsaturated hydrocarbons having nauseous odours, and Bickford (2) isolated similarly objectionable material from the condensate obtained from a commercial soybean oil deodorizer.

Removal of possible sources of flavour instability in linseed shortening was attempted by deodorizing and by winterizing refined and bleached linseed oil, before hydrogenating and deodorizing in the usual manner. Table I shows the negative results. Preliminary deodorization at 270° C. was strongly detrimental, as evidenced by a short storage life and a high mean peroxide value.

Low to High Temperature Hydrogenation

Previous investigations on the effect of different conditions of hydrogenation upon flavour stability were continued. A selective hardening of linseed oil is desirable because of the high proportion of linolenic acid that must be hydrogenated. It is also important that the formation of iso-linoleic acid be minimized, since the breakdown of this compound is considered to be a cause of flavour reversion (4).

A hydrogenation procedure in which the temperature was raised during the process from a low to a high value appeared to offer a means of hydrogenating iso-linoleic acid without building up a high concentration of saturated acids (5). A series of samples of linseed shortening was prepared in the manner previously described, employing temperatures in the range 110° to 240° C., and available commercial- and laboratory-prepared nickel catalysts (4, 6). Temperature ranges, catalysts, peroxide values (iodimetric method) averaged over the entire sampling period (56 weeks), and storage lives are given in Table I. Storage tests at 43.3° C. showed no improvement in the products obtained over the average for standard processing. Mean peroxide values were low, except in the case of one of the 110° to 160° C. samples, indicating the usual resistance to oxidation.

Hydrogenation to Various Iodine Numbers

Earlier work reported susceptibility to flavour reversion at a maximum at iodine value 80 to 130, with complete stability at iodine value 6 (4, 6). It was assumed that susceptibility varied with the amount of iso-linoleic acid present.

TABLE I

STORAGE LIFE AND MEAN PEROXIDE VALUE OF LINSEED SHORTENINGS PREPARED BY VARIOUS
PROCESSING PROCEDURES AND STORED AT 43.3° C. FOR 56 WEEKS

Group	Group variant			Storage life, weeks	Mean peroxide value*, ml. per gm.
	Alkali treatment, ° Bé	Catalyst	Temperature range, ° C.		
Low to high temperature hydrogenation	30	Standard	110–160	18	8.1
	30	Standard	110–160	29	3.7
	30	Standard	110–190	17	1.0
	30	Standard	110–190	17	3.5
	30	Standard	115–190	17	3.1
	30	Standard	115–240	19	1.0
	30	Standard	140–190	15	1.3
	30	Standard	140–235	16	0.8
	30	Commercial	110–160	25	2.5
	30	Commercial	110–190	31	1.6
	30	Commercial	110–160	17	4.4
	30	Commercial	110–190	32	2.0
	40	Standard	115–190	15	1.2
	40	Standard	115–190	6	1.3
Additional deodorization	Deodorized at 200° C. before hydrogenation			16	9.6
	Deodorized at 270° C. before hydrogenation			1	31.6
Use of winterized oil	Winterized at 0° C. after refining			18	2.6
Average for 18 samples by standard procedure				29	
Estimated necessary difference				12	

* *Iodimetric method (13).*

However, it has since been noticed that flavour reversion is often pronounced at iodine values considerably lower than those that would be expected from the theory that iso-linoleic acid is the cause of reversion. This phenomenon was investigated further.

Two hydrogenations were carried out according to the usual processing technique in a large converter, so equipped as to permit removal of samples of varying degrees of saturation while the operation continued. Samples thus obtained were deodorized, stored at 60° C., and sampled at weekly intervals for nine weeks. Composition data for the two series are shown in Table II, and storage lives and mean values for peroxide oxygen (ferrometric method), Kreis test, and fluorescence are presented in Table III (A).

It will be seen that for both series of hydrogenated oils there was no regular improvement in storage life with decreasing iso-linoleic acid concentration. There was in fact an apparent slump in flavour stability at lower iodine values, contrary to previous observations based on a rapid heat test (6). It will also be noted that storage lives in the two series were not the same for similar

TABLE II

COMPOSITION OF SAMPLES OF HYDROGENATED LINSEED OIL OF DIFFERENT IODINE NUMBERS

Time of hydrogenation, min.	Iodine number	Fat acid content*, %					
		Saturated	Iso-oleic	Oleic	Iso-linoleic	Linoleic	Linolenic
Series 1							
33	88.3	20.9	10.4	47.6	15.4	3.5	2.2
38	77.3	27.1	12.9	44.2	13.0	1.8	1.0
45	60.8	38.7	13.1	38.9	8.5	0.8	0
49	52.8	44.3	12.2	37.8	5.3	0.4	0
53	40.2	55.6	8.2	33.8	2 0	0.4	0
58	39.9	—	—	—	—	0.3	0
Series 2							
35	89.4	20.6	10.3	49.0	15.1	3.8	2.2
42	77.6	26.7	11.3	45.8	13.7	1.8	0.7
50	62.2	37.4	12.7	40 0	9.2	0.7	0
54	53.6	44.0	12.1	37.6	5.9	0.4	0
58	48.2	47.4	8.8	40.3	3.2	0 3	0
62	42.2	53.2	8.0	36 4	2.0	0.3	0

*For method of determination see Ref. (4).

TABLE III

MEAN PEROXIDE, KREIS AND FLUORESCENCE VALUES, AND STORAGE LIVES, FOR LINSEED SHORTENINGS STORED AT 60° C. FOR NINE WEEKS

Group	Group variant	Mean values of measurements on stored samples			Storage life[3], weeks
		Peroxide oxygen content*, m.e. per kgm.	Kreis value, extinction coefficients	Fluorescence, photo-fluorometer units	
A. Varying iodine number	Series 1				
	88.3	53 9	44.2	72.0	2.4
	77.3	22.8	22.0	74 0	2 2
	60.8	9.7	9.0	64.5	3.6
	52 8	9.7	8.3	60.7	2.8
	40.2	6.7	5.7	61.7	3.2
	39.9	4.5	5.9	58.4	4+
	Series 2				
	89.4	42.2	36 1	70.3	1.8
	77.6	24.3	20.5	68.6	1.8
	62.2	9.2	9.2	63 6	3.9
	53.6	8.3	7.2	60.2	3.6
	48.2	6.8	6.3	56.7	3.0
	42.2	5.9	4.8	56.2	5+
B. Commercial polymerized oil fraction	Hydrogenated	14.9	22.4	48.9[1]	0.7
	Hydrogenated	30.9	27.5	39 8[1]	0.5
	Esterified and hydrogenated	70.5	23.4	56.5[2]	1.8

* Ferrometric method (7).
[1] 1 : 50 dilution.
[2] 1 : 100 dilution.   All other fluorescence measurements 1 : 10 dilution.
[3] Estimated necessary difference: one week.

iodine values. This may have been due to different rates of hydrogenation, since the composition data do not indicate any reason for the discrepancies.

At the higher iodine values there was some increase in Kreis and peroxide values before the appearance of definite flavour reversion. This increase was steady with no definite changes in the rate of accumulation and appeared to be independent of reversion. Below iodine number 60 these values remained practically stationary. Fluorescence showed a decrease during storage but the changes were erratic throughout. Fluorescence values were generally higher at the higher iodine numbers.

Use of Polymerized Oils

In view of the stability to reversion found in hydrogenated, acetone-segregated fractions of polymerized linseed oil (9), a commercial product (1) obtained by solvent extraction of polymerized linseed oil was examined. In addition to the regular polymerized fraction, two laboratory-prepared materials (5) were hydrogenated.

The commercial process of acetone extraction of polymerized linseed oil yields an oil that is very dark in colour, and has a 'strong bodied oil' odour. The free fatty acid content is high, about 13% expressed as oleic acid, and this cannot be removed by ordinary refining methods, since emulsifying properties prevent the 'break'. Alkali refining has been found unsuccessful even after lengthy steam deodorization, so the regular commercial fraction was esterified with glycerol to reduce the free fatty acid content to 1.6%. Refractive index, linoleic acid, linolenic acid, and free acid content for all three samples are shown in Table IV. The linoleic and linolenic acid contents

TABLE IV

ANALYSIS OF SOLVENT SEGREGATED FRACTIONS OF POLYMERIZED LINSEED OIL

Sample	Refractive index at 50° C.	Linoleic acid, %	Linolenic acid, %	Free fatty acid as oleic, %
Laboratory sample	1.4736	14.4	33.0	2.1
Laboratory sample	1.4729	11.5	22.3	3 6
Regular process sample, esterified with glycerol	1.4750	9.3	8.3	1.6

of the laboratory-prepared fractions were high since these were obtained from only partially polymerized oils. However, even in the regular product the content of these acids was still appreciable.

The three samples were hydrogenated with difficulty (5). There was considerable foaming of the oils, and on deodorizing a large amount of crystalline material (acid number 126.5) tended to choke the side-arm of the deodorizing flask. The final products were dark coloured. Owing to the

presence of polymerized acids in the products, saturated acids could not be estimated by the usual method and hence the amount of iso-linoleic acid could not be determined.

The products were stored at 60° C. as outlined in the preceding section. Storage lives and mean values for the chemical tests are shown in Table III (*B*). Storage lives were short, although there was some doubt among members of the testing panel that the off-odours developed were typical reverted odours of hydrogenated linseed oil. As indicated by the high mean values for peroxide and Kreis tests these products were unstable to oxidation. Mean fluorescence was much higher than with ordinary linseed shortening.

Discussion

The experiments outlined indicate no solution to the flavour reversion problem by removal of impurities from the oil, by selective hydrogenation, or by the use of solvent-segregated material from a commercial polymerization process.

The failure of selective hydrogenation to minimize reversion may be explained in part by the finding that iso-linoleic acid is more resistant to hydrogenation than linoleic or linolenic acids (5). The use of commercial fractions of polymerized oils does not appear promising, because of their high colour and free fatty acid, and emulsifying properties. Moreover, the polymerization does not alter all the linolenic acid, and the hydrogenated products are unstable to oxidation and contain polymerized material of undetermined nature. However, it must be emphasized that the commercial process is not identical with the procedure reported by Privett, Pringle, and McFarlane (9), which yields a more acceptable product.

The storage experiments with shortenings of various iodine values suggest that iso-linoleic acid is not the only cause of reversion. They also confirm earlier views that flavour changes may occur without appreciable oxidative changes, although the vacuum storage results are evidence that some oxygen must be present for reversion to take place.

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CHARACTERISTICS OF CANADIAN LARD[1]

By H. J. Lips[2]* and G. A. Grant[3]

Abstract

Mean values for measured properties of 33 samples of lard obtained from 26 packing plants across Canada were: iodine number, 58 7; saponification number, 193.9; melting point, 43 5° C.; smoke point, 382° F.; colour, 8.8Y, 1.6R; unsaponifiable matter, 0 43%; fatty acid composition: saturated, 45.6%, oleic, 44.7%, linoleic, 8.7%, linolenic, 0.6%, arachidonic, 0 4%; storage life at 26.7° C., 9.2 weeks; Swift stability, 3.5 hr.; iodimetric peroxide, 1.6 ml. of 0.002 N thiosulphate per gm.; ferrometric peroxide, 9.7 m.e. per kgm.; Kreis test, 9.9; Stamm test, 2.3; alpha-dicarbonyl test, 3.4; free fatty acid, 0.4%; fluorescence, 79.2. The distribution of values is shown by histograms.

Simple correlation coefficients computed between measured properties showed the following to be associated: melting point with iodine number; free fatty acid content with melting point, smoke point, and red colour; storage life at 26 7° C. with log of Swift stability and initial ferrometric peroxide, Kreis, and alpha-dicarbonyl values.

Introduction

In pre-war years there was usually a lard surplus because the housewife preferred hydrogenated vegetable shortening to the animal product. The factors that have contributed to the inferior competitive position of lard are: lack of resistance to spoilage, lack of blandness, and unsuitability for use in deep-fat frying due to a low smoke point (28, 42). However, in pastry the shortening power of lard is often superior to that of hydrogenated vegetable oils (41, 58).

In the United States, progress has been made in the development of bland lards that may be kept for extended periods without refrigeration. These products owe their desirable characteristics to special processing, including: deodorization, partial hydrogenation, addition of hardened lard "flakes", or the incorporation of antioxidants such as gum guaiac and lecithin (44, 49, 61). In Canada, although the Food and Drugs Act has been amended to permit the incorporation in lard of certain antioxidants, commercial stabilization under the Act has not begun. Provided that additions are indicated on the container and that not more than 0.2% of stabilizing material is added, singly or in combination, the following substances may now be used: gum guaiac; vegetable oil containing tocopherols; lecithin; citric, tartaric and ascorbic acids (12).

Formerly Canada imported over 50% of her fats and oils requirements, exclusive of butter (35). During the war, the lard industry expanded, owing to a large increase in hog production and to emergency salvage and carcass

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trimming operations.   It is expected that there will be an eventual surplus of lard, and one of the problems confronting the Canadian packing industry will be the improvement of this product so that high levels of export and domestic consumption can be maintained in the face of post-war competition by shortenings prepared from vegetable oils.

As a basis for proposed work to improve the quality of Canadian lard, it was considered advisable to determine the characteristics of the product as freshly produced.   Surveys of Canadian lard were conducted as early as 1888 by the Food and Drugs laboratory (then a branch of the Department of Inland Revenue), but these were concerned only with adulteration of lard in respect to other fats and excess moisture (30).

A preliminary examination of market samples obtained in the city of Ottawa in the summer of 1944 showed an average keeping time of only five weeks at 26.7° C.   With this indication of stability as a guide a more extensive survey of available commercial products was begun in September, 1944.   Questionnaires concerning plant practice were submitted to Canadian packing houses, and representative samples of processed material were requested.

Materials and Methods

A total of 33 samples of lard was obtained from 26 packing plants across Canada and stored at −40° C. until required.   Of these samples 12 were dry rendered and 21 wet rendered.   As indicated in replies to questionnaires, the details of processing procedure varied considerably from plant to plant.

The product was characterized by determination of Kaufmann iodine number (14, 26); saponification number (24); capillary melting point (5); unsaponifiable matter (2); oleic, linoleic, linolenic, and arachidonic acids (9); smoke point (2); Lovibond colour (2); free fatty acid (32); and Swift stability* (27, 40).   Organoleptic storage life at 26.7° C.; iodimetric and ferrometric peroxide oxygen; Kreis, Stamm, and alpha-dicarbonyl tests; and fluorescence were determined by methods reviewed elsewhere (18).

Results and Discussion

It was necessary to respect the confidential nature of some of the data obtained from the questionnaires but the majority of the laboratory results are reported (Fig. 1 and Table I).   Initial odour is not given, but should be mentioned because many samples were objectionable in varying degree, having definite 'porky', 'tankage', or 'burnt' odours.   Lovibond blue reading and initial iodimetric peroxide content are not represented graphically, as the results included a number of zero values.   No histogram for arachidonic acid concentration is given because the variation in value was slight.

* *When the indicator method (40) is used to determine Swift stability, the indicator should be renewed after the volatile fatty acids originally present have been driven off.   This should not take longer than 30 min.*
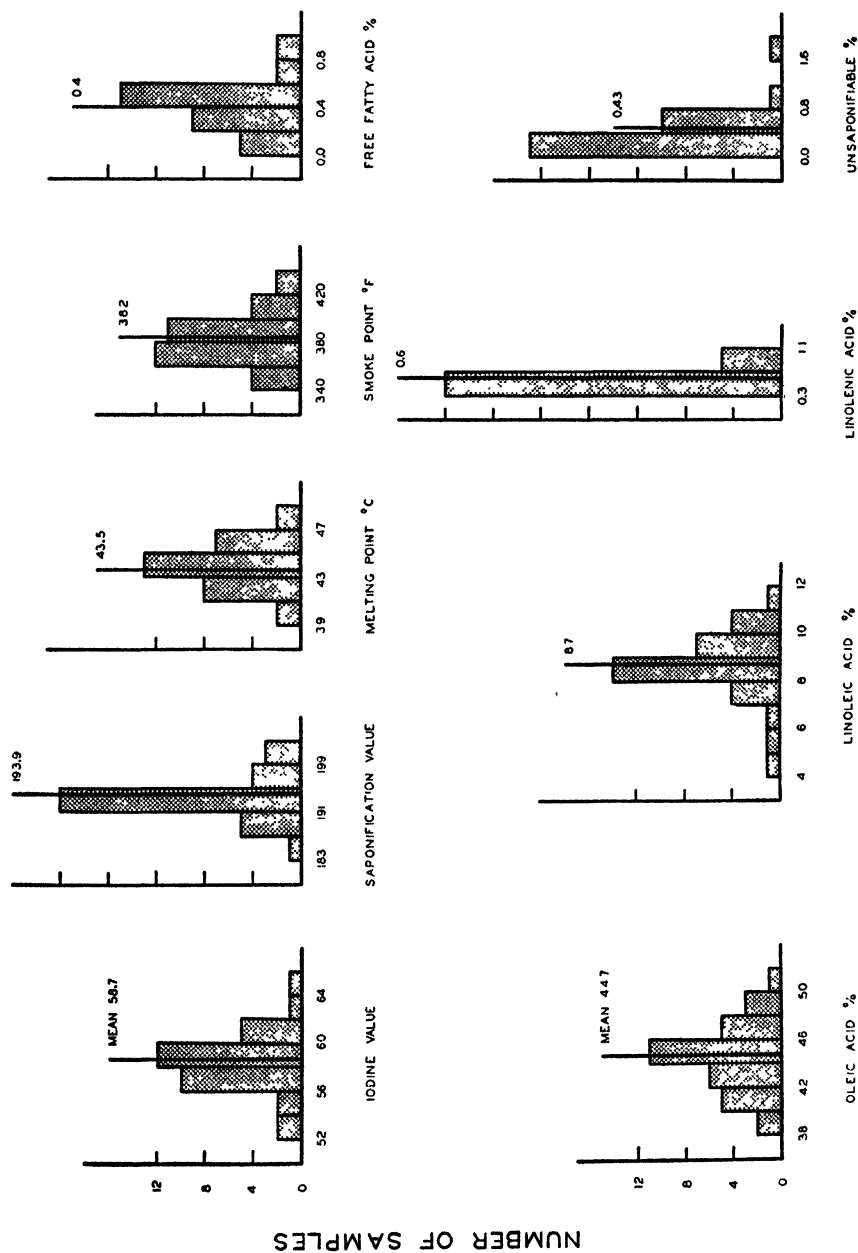
FIG. 1a. *Distribution of measured properties of Canadian lard.*
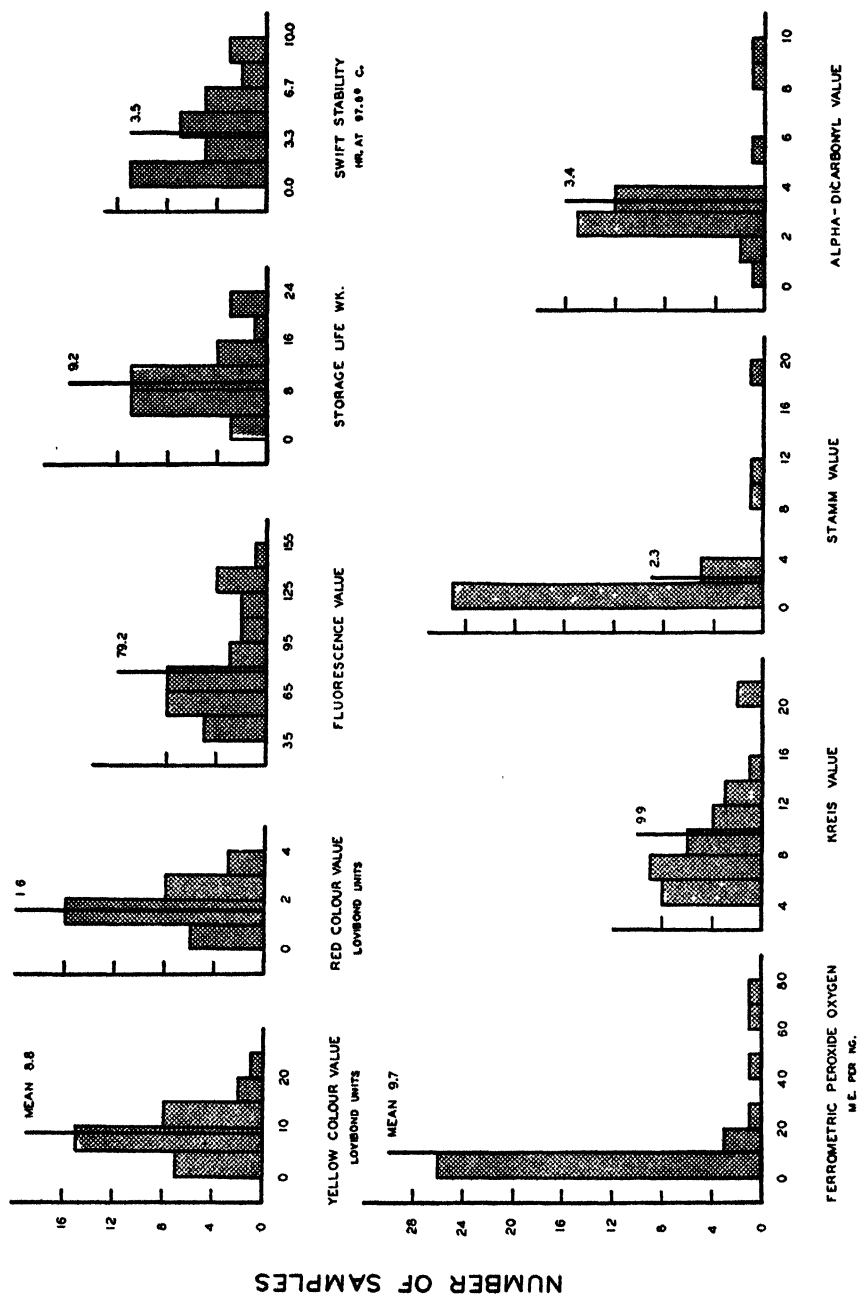
FIG. 1b. Distribution of measured properties of Canadian lard.

TABLE I

MEAN VALUES AND RANGES FOR MEASURED PROPERTIES OF CANADIAN LARD

Measurement	Mean	Range	Measurement	Mean	Range
Iodine number	58.7	53.1–65.3	Swift stability, hr. at 97.8 °C.	3.5	0.3–9.0
Saponification number	193.9	184.2–202.2	Ferrometric peroxide, m.e.		
Melting point, °C.	43.5	39.3–47.9	per kgm.	9.7	0.0–73.2
Smoke point, °F.	382	345–438	Iodimetric peroxide, ml. of		
Yellow colour, Lovibond			0.002 N thiosulphate		
units	8.8	3.0–22.3	per gm.	1.6	0.0–14.2
Red colour, Lovibond units	1.6	0.4–3.1	Kreis test, extinction coef-		
Blue colour, Lovibond units	0.2	0.0–1.2	ficients	9 9	3.7–20.7
Saturated acids*, %	45.6	—	Stamm test, extinction coef-		
Oleic acid*, %	44.7	38.6–51.8	ficients	2.3	0.0–19.9
Linoleic acid*, %	8.7	4.6–11.3	Alpha-dicarbonyl test,		
Linolenic acid*, %	0 6	0.4–1.1	extinction coefficients	3.4	0.8–9.9
Arachidonic acid*, %	0.4	0.3–0.5	Free fatty acid, as % oleic	0.4	0.1–0.9
Unsaponifiable matter, %	0.43	0.20–1.70	Fluorescence, photofluoro-		
Storage life, weeks at 26.7° C	9 2	2.0–22.0	meter units	79.2	39.8–146.0

Expressed as % of total acids.

Characteristics of Canadian Lard

Values for iodine number, saponification number, and melting point had a fairly normal distribution (Fig. 1). Variation in these values, in the absence of adulteration or advanced spoilage, would seem to be chiefly due to differences in the original fat before processing.

There was appreciable variation in oleic, linoleic, and linolenic acid concentrations as determined spectrophotometrically. Saturated acid values were obtained by difference only. Here again the differences were concerned with the original raw materials.

Values for unsaponifiable matter vary to some extent with the method of rendering the lard, and large variations may affect the values obtained for other measurements on the whole fat (36, pp. 401, 425). The values greater than 0.8% are unusually high.

It is considered that the smoke point of lard should be at least 400° F. to compete with that of shortening, since a survey of 17 wartime shortenings, including animal–vegetable compounds, showed a mean value of 424° F. (39).

For several samples it was necessary to remove suspended material by filtration before colour measurements could be made. Blue readings were obtained for 10 of the 33 samples, and it was found that the presence of carbon in suspension markedly increased the apparent blue colour. Variation in colour would appear to be chiefly governed by factors of processing technique.

Stability of most of the lard samples was poor, as estimated by storage life at 26.7° C. and by Swift stability at 97.8° C. The magnitude of these measurements depends on the nature of the original material and the details of processing procedure, if it is assumed that no antioxidants were used.

The values for iodimetric peroxide, ferrometric peroxide, Kreis test, Stamm test, alpha-dicarbonyl test, and free fatty acid reflect the degree of exposure of the lard during processing to spoilage by biological and chemical action. Considerable variation in these respects is shown in Table I and Fig. 1. Fluorescence values were unevenly distributed.

Interrelation of Measured Properties

The literature contains a number of references to relations between various measured properties of fats and oils. Iodine value is reduced by marked oxidation (33, p. 95; 36, p. 425), saponification number is influenced to some extent by free fatty acid content (36, p. 394), and melting point is appreciably altered by changes in free fatty acid (36, p. 329). Moisture content, by affecting lard transparency, may influence estimation of the capillary melting point (17; 36, p. 288). However, according to the Canadian Food and Drugs Act (12), moisture content of lard is limited to 1%, and the product must be free from rancidity. In the absence of changes in composition, smoke point varies inversely with free fatty acid content (7; 23, pp. 132-136).

In the present study the relation of measured properties to one another was investigated by calculating simple coefficients of correlation, except where this was precluded by the presence of a large number of zero values, as in the case of iodimetric peroxide and Lovibond blue measurements. Most of the correlations proved to be insignificant; only the more important ones are reported (Table II).

Iodine number was inversely related to melting point, but no association of saponification number with other measured properties was found. The significant association of melting point with free fatty acid content indicates that the free fatty acids formed before or during processing lowered the melting point.

Smoke point, Lovibond red colour, and free fatty acid content were all interrelated. High values for free fatty acid were associated with low smoke point and high red colour. Lowering of the smoke point by free fatty acids is a well recognized effect, and significant correlations have been reported (42). The relation of free fatty acid to red colour suggests that there was a parallel increase in these two measurements during processing. Red and yellow colour readings were directly related.

The correlation of storage life at 26 7° C. with log Swift stability (Fig. 2) indicates that the latter determination has certain usefulness in the prediction of lard keeping quality at lower temperatures, at least in the absence of added antioxidants. There has been some question about this point (51). Storage life varied inversely with ferrometric peroxide, Kreis and alpha-dicarbonyl values; that is, samples with high values for these chemical measurements had short storage lives, as oxidative deterioration was already in progress. Iodine value, content of saturated and unsaturated acids and unsaponifiable matter, and fluorescence had no observed important relation to storage life. The results support a previous suggestion that degree of

unsaturation in pigs' fat is not necessarily the limiting factor in determining stability (34, pp. 60-64).

Other relations examined between measured properties were not significant.

TABLE II

COEFFICIENTS OF CORRELATION BETWEEN MEASURED PROPERTIES
OF CANADIAN LARD

Quantities correlated	No. of values	Correlation coefficient
Iodine number with:		
Melting point	33	− .41*
Melting point with:		
Smoke point	33	.41*
Lovibond yellow	33	− .29
Lovibond red	33	− .24
Free fatty acid	33	− .59**
Smoke point with:		
Lovibond yellow	33	− .19
Lovibond red	33	− .38*
Free fatty acid	33	− .87**
Lovibond yellow with:		
Lovibond red	33	.82**
Lovibond red with:		
Free fatty acid	33	.41*
Storage life with:		
Log Swift stability	29	83**
Ferrometric peroxide	26	− 63**
Kreis test	29	− 39*
Stamm test	27	− .28
Alpha-dicarbonyl test	27	− .36*

* Indicates 5% level of statistical significance.
** Indicates 1% level of statistical significance.

Relation of Measured Properties to Plant Practice

Stability and smoke point were considered likely to be altered by variations in processing procedure, so the effect of these variations upon storage life and smoke point was assessed on wet and dry rendered lards considered together and separately (Table III). However, the calculated correlation of any one plant practice with the measured properties is not strictly valid, as all other factors were not constant among plants. Moreover the calculations are based on a limited number of values, and the results may not reflect general operational experience in all cases.

A preliminary analysis of variance demonstrated no important difference in mean storage life between wet and dry rendered lard, but showed that the mean smoke point for dry rendered lard was significantly higher than for the wet rendered product. The latter result is attributable to the higher free fatty acid content of the wet rendered fats, due to their exposure to the hydrolysing effect of long contact with steam or hot water.
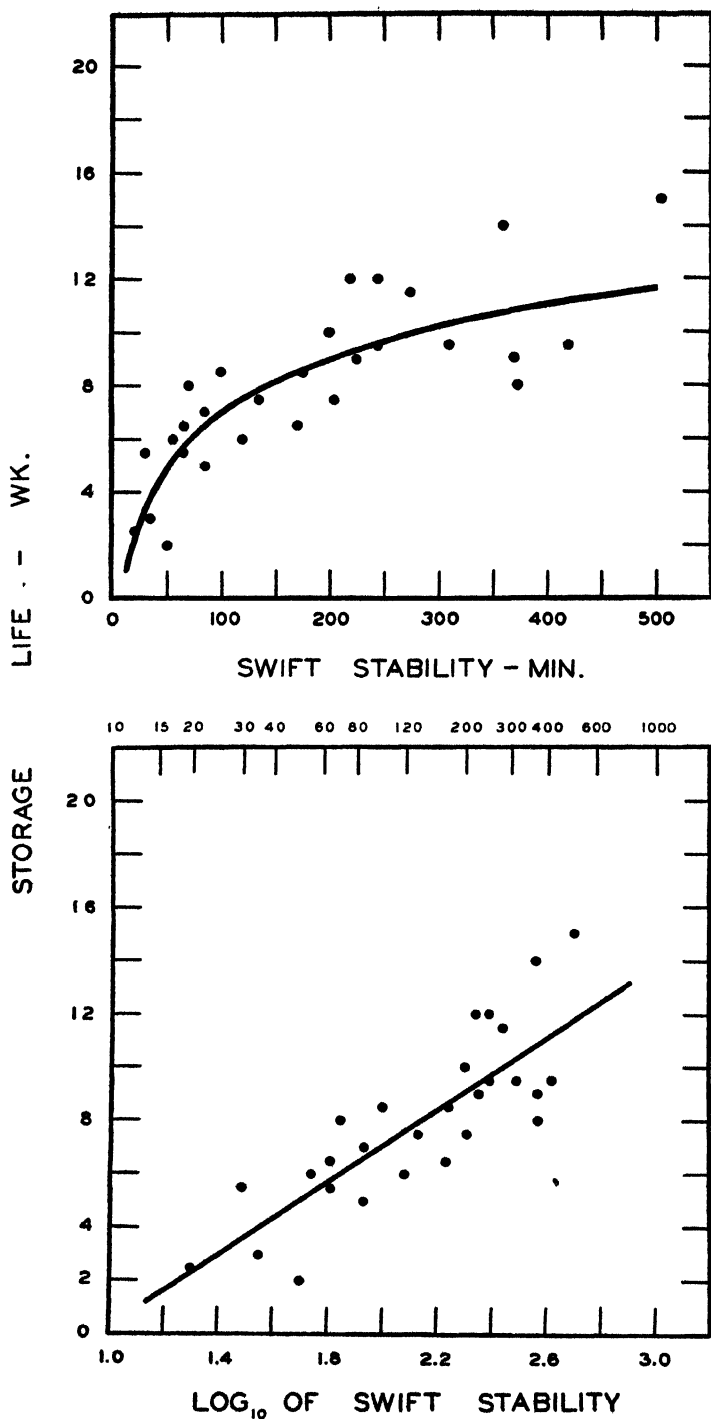
FIG. 2.   *Relation of Swift stability at 97.8° C. to storage life at 26.7° C. for Canadian lard.*

TABLE III

COEFFICIENTS OF CORRELATION BETWEEN PLANT PRACTICES AND MEASURED PROPERTIES FOR
CANADIAN LARD

Quantities correlated	No. of values			Correlation coefficients		
	Wet rendered lard	Dry rendered lard	Total	Wet rendered lard	Dry rendered lard	Total
Storage life with:						
Killing fat, %	21	12	33	.15	.10	.14
Cutting fat, %	21	12	33	— .14	— .35	— .23
Bones, %	21	12	33	.77**	— .15	— .22
S.P.† material, %	21	12	33	— .02	— .31	— .11
Rendering temperature	21	12	33	.41	— .30	— .12
Rendering time	3	11	14	— .42	.69*	— .02
Deodorizing temperature	—	4	5	—	.00	— .40
Deodorizing time	—	4	5	—	— .70	.08
Filtering temperature	20	11	31	— 31	— .43	— .32
Time, processing to packaging	21	12	33	.10	— .30	— .12
Smoke point with:						
Killing fat, %	21	12	33	— .77**	.52	— .19
Cutting fat, %	21	12	33	— .15	.22	— .09
Bones, %	21	12	33	.40	.53	.33*
S.P.† material, %	21	12	33	— .03	.20	.11
Rendering temperature	21	12	33	.33	.82**	.51**
Rendering time	3	11	14	.99*	— .56	— .71**
Deodorizing temperature	—	4	5	—	— .88	— .45
Deodorizing time	—	4	5	—	— .83	— .42
Filtering temperature	20	11	31	.32	— .12	— .22
Time, processing to packaging	21	12	33	— .10	.19	— .08

† *Sweet pickle.*
* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

The storage life of wet rendered lard appeared to be lengthened by increase in amount of bones (Table III), presumably because of their content of natural stabilizing substances. The presence of 0.14% lecithin in pig-bone fat as compared to 0.04 to 0.06% in lard has been reported (25). With the dry rendered lard, increase in rendering time appeared to be beneficial. This may be related to better extraction of natural stabilizers from the non-fatty material. Although correlations of storage life with filtering temperature failed to attain statistical significance, they were all negative, indicating that moderately low filtering temperatures (160° to 170° F.) would be desirable for the production of more stable lard.

High smoke point was favoured by decrease in killing fat in wet rendering, and by an increase in bones. A negative correlation of smoke point with cutting fat rather than killing fat had been anticipated, since the former fat usually has more time to develop free fatty acids by enzymic action before it is rendered (23, pp. 132–136; 37, 45, 60). Similarly it was expected that

increase in bones might lower the smoke point, since these may stand for some time before rendering, with consequent development of free fatty acids in the fat that they carry. No satisfactory explanation can be offered for these discrepancies at present because of limited information about the operations concerned. The relation between smoke point and rendering temperature was positive, and between smoke point and rendering time negative. This suggests that rendering should be carried out at a relatively high temperature in as short a time as possible (23, pp. 132–136; 43, 55).

Although none of the other relations tabulated was significant, reduction in deodorizing temperatures and times used appeared to be desirable. In general the influence of processing procedures upon wet and dry rendered products was dissimilar.

Comparison with Lards from Other Countries

Extensive control examinations of lard in other countries have been reported (1, 13).

Comparative figures in the literature on the composition of lard are largely based on chemical rather than spectrophotometric measurements. For 27 American lards (4), the calculated compositions were predicated on the absence of fatty acids more unsaturated than linoleic (Table IV). However, the presence of linolenic, unsaturated C_{20-22}, and conjugated acids has been demonstrated (10; 31; 21, pp. 80–86). The presence of arachidonic acid is used as the basis of a spectrophotometric method for distinguishing between lard and hydrogenated vegetable oils (6). The greater unsaturation of other lards as compared to Canadian lards is undoubtedly due in part to differences in the nature and fat content of the diet of the hog (33, pp. 17–26). Changes in the diet similarly affect melting point, but have less effect on saponification number.

Comparative figures for lard colour have not been found in the literature. Authentic blueness in lard is said to be due to a natural pigment, and is considered an indication of careful processing rather than poor handling.* The blue pigment is unstable, and on oxidation it changes to red, or a mixture of red and yellow (23, pp. 157-158). Its presence is objectionable, since the consumer prefers whiteness.

The smoke point figure quoted in Table IV for American samples does not include the value given for a bland lard (59).

Available figures for small numbers of American samples indicate that lard usually has indifferent keeping quality unless it is stabilized by the addition of antioxidants, or subjected to special processing treatments (50).

The use of fluorescence in the study of fats to detect impurities and deterioration has been suggested by several workers (19, 20, 47, 54) but no recent data are available for comparison. Fluorescence in lard is said to depend both on state of preservation and manner of processing (8, 16, 48, 53).

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* *Anon. National Provisioner, 112(20): 15. 1945.*

TABLE IV

A COMPARISON OF VALUES FOR MEASURED PROPERTIES OF CANADIAN LARD WITH OTHER
REPORTED AND RECOMMENDED VALUES

Measurement	Comparative values			
	Canadian		American	Other
	Mean	Range		
Iodine number	58.7	53.1 65.3	27 samples, 64.6 (4) Recommended, 46–70 (3)	General, 46–66 (22, p. 136) English, 108 samples, 57–73 (56) Portuguese, 30 samples, 63.9 (46)
Saponification number	193 9	184.2 202 2	Recommended, 195–202 (3)	General, 193–200 (22, p. 136) Portuguese, 30 samples, 195.8 (46)
Melting point, °C.	43.5	39.3–47.9	—	General, 28–48 (22, p. 136)
Smoke point, °F.	382	345 438	6 samples, 371 (59)	—
Saturated acids*, %	45 6	—	27 samples, 36 8 (4)	Portuguese, 30 samples, 38 5 (46)
Oleic acid*, %	44.7	38.6–51.8	27 samples, 51 5 (4)	Portuguese, 30 samples, 49 2 (46)
Linoleic acid*, %	8.7	4.6 11 3	27 samples, 11 7 (4)	Portuguese, 30 samples, 12.5 (46)
Arachidonic acid*, %	0 4	0.3 -0 5	0 02–0 21 (15) 0.31–0 40 (11) 0.2 -0 6 (6)	
Unsaponifiable matter, %	0 43	0 20–1 70	Recommended, 1 0 max. (3)	General, 0 2–0 4 (22, p 136, 29, p. 135) Portuguese, 30 samples, 0.14 (46)
Swift stability, hr. at 97.8 °C.	3.5	0.3–9.0	7 samples, 5.3 (50)	Other Canadian, 14 samples, 2.2 (38, 40)
Iodimetric peroxide, ml. of 0.002 N thiosulphate per gm.	1.6	0.0–14.2	4 samples, 1.5 (28) 6 samples, 0.7 (52) Recommended, 2.5 ml. max. (3)	—
Free fatty acid, as % oleic	0.4	0 1–0.9	6 samples, 0.26 (59) 5 samples, 0 29 (28) 3 samples, 0.33 (57) 4 samples, 0 35 (28) Recommended, 1% max. (3)	General, 0.2 0.7 (22, p 136)

* *Expressed as % of total acids.*

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PRESERVATION OF EGGS

VI. EFFECT OF VARIOUS OILS AND OILING TEMPERATURES ON THE KEEPING QUALITY OF SHELL EGGS STORED AT 70° F. AND 30° F.[1]

By N. E. Gibbons,[2] Ruth V. Michael,[3] and Ursula Irish[3]

Abstract

The quality of eggs oiled when one day old with eight commercial oils and six mixtures and stored at 70° and 30° F. was assessed by candling, yolk index, and thick white height. Mineral oils with Saybolt viscosities of 70/100 at 100° F. maintained egg quality and prevented weight loss better than oils of lower viscosity. Viscosity of the oil seemed more important than pour point, although at 30° F. there was some indication that a high pour point was advantageous. Oiled eggs maintained their grades two to three times longer than unoiled eggs and lost from $\frac{1}{10}$ to $\frac{1}{4}$ as much weight. Heavy oil diluted with mineral spirits did not give as good results as lighter oils of comparable viscosity. The addition of vaseline and magnesium stearate improved the action of the light oils.

There was little difference in the quality of eggs dipped into oils maintained at 76°, 100°, and 130° F.

Introduction

In 1944 the British Ministry of Food requested the shipment of shell eggs to Britain and suggested that eggs placed in storage for shipment in the fall should be oiled. Although there are many reports on oils used for oiling eggs in the United States (e.g. 3, 6, 8, 9), little has been done in Canada. There is also some question as to whether the eggs should be oiled in cold or warm oil.* The present investigation was undertaken to determine which were the most suitable of the available oils and the effect of different temperatures of oiling.

Materials and Methods

The oils usually recommended for eggs are white mineral oils with low viscosities and high pour points. Oils of this type were obtained from a number of firms. Their characteristics as reported by the manufacturers, and as determined in these laboratories and by the N.R.C. oil testing laboratory, are given in Table I.

The main function of the oil is to seal the shell against water loss. The prevention of loss of carbon dioxide would also seem desirable so that the addition of substances more impervious to gas was indicated. Vaseline makes an excellent preservative but the thick films are messy and unsightly (7). In an attempt to seal the shell without the disadvantages of thick films one of the oils (G) was fortified with vaseline, or with magnesium stearate plus

TABLE I

PHYSICAL PROPERTIES OF OILS USED, ARRANGED IN ORDER OF DECREASING VISCOSITY

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
Laboratory findings								
Viscosity, Saybolt								
Universal sec., 100° F.[1]	100	89	81		60	59	52	51
Sp. gr., 60° F./60° F.	875	.850	.847		833	.833	.833	830
Pour point, °F.	5	15	10		45	45	30	35
Flash point (O.C.), °F.	375	385	370		340	340	320	320
U.S.P. acid test	Fail	Pass	Pass	Pass	Fail		Pass	Pass
Viscosity, S.U. sec., 76° F.	168	120	109	98	79		64	63
100° F.	101	87	82	69	60		52	51
130° F.	76	63	60	55	50		44	44
Manufacturers' specifications								
Sp. gr., 60° F./60° F		850/ 860	835/ 845	835/850	825/ 840		820/ 830	825/ 835
Viscosity, S.U. sec., 100° F.	77/82	85/90	70	70/80	50/60	50/60	50/60	50/55
Pour point, °F.	25	30	25	45	45	45	40	35
Flash point, °F.	355	345	310	345	325		300	280
Fire point, °F.	385	395	350	395	365			
Colour, Saybolt	30+	30+	30+	30+	30+		30+	30+

[1]*Calculated from centistokes.*

Nacconal. A heavy oil (350 viscosity) was diluted with an odourless kerosene in the proportions of 60:40 and 70:30. The viscosities of these mixtures are given in Table II.

TABLE II

SAYBOLT VISCOSITIES AT 76°, 100°, AND 130° F. OF THE OIL COMBINATIONS USED

		Viscosity at:		
		76° F.	100° F.	130° F.
I	350 oil + kerosene 70 : 30	102	76	55
J	350 oil + kerosene 60 : 40	72	55	45
K	Oil *G* + 7% vaseline	69	58	46
L	Oil *G* + 5% vaseline	68	53	45
M	Oil *G* + 0 2% magnesium stearate + 0 015% Nacconal	66	53	44
N	Oil *G* + 0.3% magnesium stearate + 0.015% Nacconal	64	52	44

The eggs were obtained from one producer who maintains a large, well managed flock of White Leghorns to which no new blood has been added for a number of years. The eggs were gathered in the morning, cooled for a few hours, packed in new Keyes trays and cases, and shipped so as to arrive at the laboratory the next morning. On receipt, they were candled, and cracked and off-grade eggs discarded. The eggs had not been cleaned and some had small spots of faecal contamination; the most noticeable of these were brushed off. The majority of the eggs were large but some of medium

size were distributed at random throughout the trays. Oiling was begun as soon as the eggs reached 50° F. to avoid surface moisture (dew point 45° F.). They were therefore oiled when one day old.

The oil was applied at the three temperatures commonly used; room temperature (in this case 76° F.), 100° F., and 130° F. The eggs were dipped into the oil for a few seconds, drained for approximately two minutes, and returned to the original trays.

The eggs were weighed in tray lots of 30 eggs in a clean tray before oiling, and after oiling and draining overnight, to determine the amount of oil retained on the shell. They were also weighed in lots of six for determination of weight loss.

Two lots of eggs were received and oiled on successive days. Those used as controls were not broken out until the day after the others had been oiled, i.e. they were two days old. Two dozen were examined from each lot and the average of these was taken as the initial value.

The eggs were stored in wooden export cases at 70° F. (21° C.) (about 40% R.H.*) and at 30° F. (−1.1° C.) (about 55% R.H.). The eggs stored at 70° F. were examined after 4, 6, 8, 10, and 12 weeks; those at 30° F. were examined after 8, 16, 24, 32, and 40 weeks.

At each sampling, one dozen eggs were removed, candled, and broken out on a glass plate to check quality. Egg quality was assessed by candling, the height of the thick albumen (10), the yolk index (5), and the pH of the mixed thick and thin white. The pH was measured with a Beckman pH meter and the instrument was frequently checked with a standard 7.8 buffer.

Results

Effect of Oils

At both storage temperatures candling results showed that the most noticeable effect of oiling was the prevention of evaporation. Usually the range allowed in the size of the air cell corresponds to the yolk shadow standards found in each grade (2). However, in oiled eggs evaporation was so slow that yolk shadow was the only criterion by which grade could be really assessed. At 70° F. control eggs had large air cells (Grade *C*) in six weeks whereas all oiled eggs at 10 weeks had air cells $\frac{3}{16}$ in. or less in height (Grade *A*). With some oils this still held true at 12 weeks. By this time eggs treated with the poorest oils and mixtures graded *B* on air cell size. In spite of the slight amount of evaporation, the yolk shadows became more distinct on storage. Most of the controls were graded *C* on yolk shadow at six weeks, whereas most of the oiled eggs were graded *A* and *B*. After 10 weeks' storage, the controls were 'no grade' and the oiled eggs were practically all Grade *C* on the basis of yolk shadow. Of the 504 oiled eggs examined after 12 weeks' storage only nine were classed as no grade before the lamp.

* *R.H.* = *relative humidity.*

At the lower storage temperature the control eggs were graded B after 16 weeks' storage and C after 40 weeks. The majority of the oiled eggs were still Grade A after 32 weeks. At 40 weeks the air cells were on the border line between Grade A and B but based on yolk shadow most of the eggs were Grade B. Actually, of 504 eggs, 135 were graded as A, 362 as B, six as C, and one as no grade.

The value of oiling in maintaining quality at 70° F. is also apparent from the results presented in Table III. After 12 weeks the weight loss with the best oils (A, B, C, D) is $\frac{1}{10}$ or less of that of the unoiled controls while with the poorest oil it is $\frac{1}{4}$ of that of the controls. The yolk index (with the exception of Oil G) and white height after 12 weeks' storage is greater in the oiled eggs than in the controls after only four weeks' storage.

TABLE III

QUALITY TESTS ON OILED AND UNOILED EGGS AFTER 4 AND 12 WEEKS' STORAGE AT 70° F.
AVERAGE VALUES PER EGG OVER THREE OILING TEMPERATURES. OILS ARRANGED
IN ORDER OF DECREASING VISCOSITY

Oil	Weight loss, gm.		Yolk index		Thick white height, in.		pH	
	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.
Unoiled[1]	3.64	11.80	.296	.174	.10	.08	9 29	9.30
A	0.29	1 00	.394	.346	.18	.16	8 36	8 20
B	0.38	0.87	.398	.357	.19	.15	8 32	8.30
C	0.41	0 81	.404	.352	.18	.16	8.30	8.25
D	0.22	1.05	.398	.348	19	.18	8 35	8.30
E	0.27	1.24	.402	.343	.18	.16	8.40	8.48
F	0.40	1.14	.400	.339	.18	.15	8.34	8.39
G	0.41	2.70	.403	.281	.19	.12	8.43	9.24
H	0.48	1 90	.396	.305	.18	.14	8.46	8.60
I	0.26	1.25	.415	.333	.19	.14	8.31	8.32
J	0.66	2.66	.410	.288	.18	.12	8.59	8.82
K	0.38	1.44	.406	.336	.19	.16	8.40	8.64
L	0.40	1.46	.418	337	.19	.15	8.36	8.62
M	0 39	1.56	.403	.339	.20	.16	8.33	8.44
N	0.34	1.38	.407	.341	.19	.15	8.28	8.54
Necessary difference[2]	0.14		0.020		0.017		0.21	

[1] *Original values—yolk index, .454; thick white height, 0.26 in.; pH 8.68.*
[2] *Necessary difference applies to oiled eggs only.*

The original pH value is high as the eggs were not examined until the day after the others had been oiled. The true original value is probably between this value and that given for the oiled eggs at four weeks. With the poor oils (G and H), carbon dioxide was apparently lost throughout the test and the pH increased with time. In all of the others there seemed to be a retention of carbon dioxide and the pH decreased until the 8th or 10th week of storage and then increased.

The analysis of variance of these results (Table IV) indicates that most of the variance due to oils can be attributed to the poor oils and mixtures (G, H, J). Much of the remaining variance is no doubt due to Mixture I. From the necessary differences (Table III) it will be seen that when the above four oils are removed there is no significant difference between yolk indices with any of the other oils and little of significance with white height. With weight loss and pH the oils fall into two groups, Oils A, B, C, and D giving better results than the other oils and mixtures.

TABLE IV

ANALYSIS OF VARIANCE ON DATA FOR OILED EGGS ONLY, STORED AT 70° F.

Source of variance	Degrees of freedom	Weight loss, mean square	Yolk index, mean square	White height, mean square	Degrees of freedom	pH, mean square
Oils	13				13	
G, H, J vs. others	1	158,383**	21,249**	122 67**	1	29,544**
Residual	12	4513**	289*	2.60*	12	679**
Temperature of oiling	2	409	286	0 18	1	1585**
Storage time	4	77,055**	33,894**	109 83**	4	3774**
Oils × temp.	26	616**	275*	3 84**	13	184
G, H, J vs. others × temperature	2	270	53	5.30	1	55
Residual	24	645	293	3 72	12	195
Time × oils	52				52	
G, H, J vs. others × time	4	11,923**	2722**	9 74**	4	1305**
Residual	48	836**	192	1 67	48	294**
Time × temp.	8	384	397*	0.58	4	279
Oils × time × temp.	104	301	156	1.13	52	113

*Exceeds 5% level of statistical significance.
**Exceeds 1% level of statistical significance.

Heavy oil diluted with mineral spirits did not give as good results as the lighter oils of comparable viscosity. The addition of vaseline and magnesium stearate improved the action of the light oil used but not to the level of the better oils.

The results of storing at 30° F. are with few exceptions much the same as at the higher temperature (Table V). With Oil C there was a greater loss in weight between the 32nd and 40th week than was expected. This was noticeable in each of the three lots of eggs oiled at different temperatures. Oils D, E, and F, which have high pour points, preserved quality better at this temperature than at 70° F. Oil H was also much better at the lower temperature.

As at the higher temperature, the weight loss with the oiled eggs was from $\frac{1}{10}$ to $\frac{1}{4}$ of that of the controls. In all but two instances the total loss over 40 weeks of storage was less in the oiled eggs than in the control eggs after eight weeks. The yolk index and white height of the oiled eggs at 40

TABLE V

QUALITY TESTS ON OILED AND UNOILED EGGS AFTER 8, 24, AND 40 WEEKS AT 30° F.
AVERAGE VALUES PER EGG OVER THREE OILING TEMPERATURES.
OILS ARRANGED IN ORDER OF DECREASING VISCOSITY

Oil	Weight loss, gm.			Yolk index			Thick white height, in.			pH		
	8 wk.	24 wk.	40 wk.	8 wk.	24 wk.	40 wk.	8 wk.	24 wk.	40 wk.	8 wk.	24 wk.	40 wk.
Unoiled[1]	1.46	4.27	7.20	.440	.404	.390	.21	.20	.20	8.86	8.88	9.01
A	0.07	0.43	0.71	.456	.449	.441	.22	.20	.21	8.12	8.06	8.29
B	0.23	0.42	0.77	.449	.457	.437	.23	.21	.20	8.00	8.04	8.30
C	0 22	0.50	1 11	.452	.439	.435	.23	.21	.20	8.14	8.10	8.25
D	0 14	0.55	0.95	.451	.451	414	.23	.21	.21	7.98	7.98	8 56
E	0.21	0.47	0.84	.479	.444	.444	.24	.22	.22	8.24	7.98	8 22
F	0.18	0.44	0.77	.439		.431	.22	20	.21	8 04	8.02	8.06
G	0.25	0.82	1.73	.442	.437	.407	.22	.20	.19	8.21	8.44	8.70
H	0 11	0.52	0.89	.456	.438	.450	.23	.20	.22	8 22	8.26	8.30
I	0.19	0.96	1.04	.457	.430	.413	.23	.21	.21	8.20	8.22	8.35
J	0.25	1.29	1.66	.447	.418	.416	.23	.19	.21	8.32	8.68	8.60
K	0.11	0 41	0.71	.442		.434	.23	.21	.23	8.12	8.22	8.21
L	0.18	0 42	0 90	.446	.453	.439	.23	.21	.23	8.11	8.19	8 32
M	0.19	0.45	1.04	.457	.444	.429	.24	.24	.23	8.14	7.96	8 46
N	0.13	0.56	0.84	.434	.444	.429	.23	.21	.21	8.02	8.24	8.38
Necessary difference[2]		0.17			0.016			0.02			0.16	

[1] *Original values—yolk index, .454; thick white height, 0.26 in.; pH 8.68.*

[2] *Necessary difference applies to oiled eggs only.*

weeks were as good as those of the unoiled eggs at 16 weeks. The final pH of all oiled eggs was much lower than that of the controls at eight weeks.

Effect of Oiling Temperature

During storage at 70° F. no significant effects of temperature of the oil at time of oiling could be found on white height, yolk index, or weight loss (Table IV). The pH was followed only in eggs oiled at 76° and 130° F.; eggs oiled at the lower temperature maintained a significantly lower pH throughout the storage period.

With eggs stored at 30° F. the temperature at which the oil was applied had no significant effect on weight loss, yolk index, or pH (Table VI). There was a significant effect with white height, the height being slightly lower in the eggs oiled at 76° F.

Amount of Oil Retained by the Eggs

The amount of oil retained by the eggs averaged from 1.0 to 2.4 gm. per tray of 30 eggs (each figure being the average of six trays), or 0.03 to 0.08 gm. of oil per egg. This is in agreement with Mallman's finding (6) of 0.06 to 0.07 gm. per egg. There was no apparent relation between amount of oil retained and the viscosity or temperature of the oil. It should be noted that these results were obtained after 24 hr. With the usual commercial equipment it has been found that it requires about twice as much oil having a viscosity of 70/90 as oil with a viscosity of 50/70. Warming the oil effects some saving.

TABLE VI

ANALYSIS OF VARIANCE ON OILED EGGS ONLY, STORED AT 30° F.

Sources of variance	Degrees of freedom	Weight loss, mean square	Yolk index, mean square	White height, mean square	Degrees of freedom	pH, mean square
Oils	13	6173.7**	666.8**	3.46**	13	1668.5**
Temp. of oiling	2	270.5	7.0	9.00**	1	236.0
Oils × temp.	26	160.2	152.9	1.65	13	71 2
Time	4	44,348.5**	3572.7**	26 50**	4	2622.0**
Oil × time	52	599.9**	271.5**	2.92**	52	286.5**
Temp. × time	8	130.2	131.0	3.63*	4	45.5
Oil × temp. × time	104	104.6	100.5	1.64	52	62.2

  * *Exceeds 5% level of statistical significance.*
 ** *Exceeds 1% level of statistical significance.*

Discussion

The eggs used in this experiment were one day old when treated, which is said to be the ideal time for maintaining quality by oiling (4). The results indicate that oils with a Saybolt viscosity of 70 to 100 at 100° F. are more suitable for maintaining egg quality than the lighter oils. In general pour point was not as important as viscosity; at 30° F. however, a high pour point seemed to be of some advantage. The use of oil at temperatures higher than room temperature offered no advantage in preserving quality or in the amount of oil retained.

These findings are in the main in agreement with previous reports. Evans (3), using oils at about 70° F., found that those of higher viscosity preserved interior quality better than the less viscous ones. When applied at 239° F., oils with a viscosity of 134 Saybolt seconds were found to be more effective than those with a viscosity of 44 (1). Although in the present experiments the oils were used at much lower temperatures, the conclusions are similar. When a light oil was used, oiling at 60° F. was said to be better than oiling at 80° F. (9). The results presented here show that there was little difference between light and heavy oils applied over a much wider range of temperature. The use of oils at elevated temperatures seems to be a trade practice originating in a desire to save oil.

Although it seems reasonable that pour points above the storage temperature should give better results (8) the present work does not altogether support this claim. Some of the best oils had very low pour points according to the laboratory tests, and even according to the manufacturers' specifications the pour points were lower than the storage temperature. However oils with the same viscosity and different pour points were not available for comparison. Judging from the data presented in the above mentioned paper (8) there is an interrelation between viscosity and pour point.

Others (3) have noted that the reduction of the viscosity of an oil with solvents decreases its efficiency as an egg preservative; an oil having an original viscosity of 75 was diluted with 10% solvent to give a mixture with a viscosity of 58 to 62 Saybolt sec. at 100° F. The results were similar to those reported above (Table III, Oil *I*) when an oil with a viscosity of 350 was diluted to a final viscosity of 76.

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PACKAGING

VII. WATERPROOFING OF FIBREBOARD CONTAINERS

By C. G. Lavers

PACKAGING

VII. WATERPROOFING OF FIBREBOARD CONTAINERS[1]

BY C. G. LAVERS[2]

Abstract

An attempt was made to find a material in which domestic fibreboard cartons could be dipped to produce waterproof packages. The waterproofing agents tested included vinyl resins, cellulose acetates, nitrocelluloses, polystyrene, chlorinated rubber, corn protein – resin, a wood oil drier combination, an alkyd, and microcrystalline wax. Agents that did not coat the surface of the box, or gave only a very light coating, were not satisfactory. Wax could not stand rough handling at low temperatures. Two nitrocellulose formulations and one vinyl resin solution were found to be particularly suitable for this application. One of the nitrocellulose waterproofing agents dried at room conditions in 15 min., and boxes coated with it withstood water immersion for over 30 days after rough handling at −40° F. This method of producing waterproof packages appears to be quite practical.

Introduction

During the war, because of uncertain conditions of handling and warehousing, many tons of warlike stores had to be shipped in waterproof containers. One of the most common methods employed in making waterproof packages was by wrapping the product to be protected, either before or after placing it in a carton, in Grade *C* material (Cellophane laminated to scrim cloth and coated on both sides with microcrystalline wax), sealing by dipping in wax, and overwrapping with kraft paper to prevent packages from sticking together. This method was expensive, and labour and time consuming. Hence, it was felt desirable to attempt to find a material in which domestic fibreboard cartons could be dipped to produce a waterproof package that would require no further processing. Several commercial waxy and resinous products were tested for this application, and this paper presents the results.

With the cessation of hostilities and consequent improvement in shipping conditions, the need for waterproof packages was greatly reduced and hence this project was not continued. For this reason, the results presented are not as complete as they might otherwise have been; however, they do serve to indicate the performance that may be expected from various types of waterproofing agents and also show the practicability of this method of packaging.

Materials and Methods

It was believed that a material for the purpose described above should have as many of the following properties as possible. It should preferably be applied without heating; should dry rapidly; should require no overwrap

to prevent packages from sticking together (blocking); should retain flexibility at low temperatures; and it should not soften and run off the package at high temperatures (140° F.).

The materials tested were of three general types: solvent deposited resins that left a surface film over the entire package, impregnating materials that left little or no surface coat, and wax. The agents tested included: vinyl resins, cellulose acetate, nitrocelluloses, polystyrene, chlorinated rubber, corn protein – resin, a wood oil drier combination, an alkyd, and microcrystalline wax. Further details are given in Table I.

An initial test of all samples was done as follows. Regular flat folding cartons with full overlapping long flaps (0.02 in. chip board; dimensions, 4 × 2¾ × 1 15/16 in.; opening end, 2¾ × 1 15/16 in.) were filled with sawdust and closed with silicate glue. Seams around the top and bottom, and at the side, were sealed with a waterproof pressure sensitive tape, and the box was then dipped in the waterproofing agent under test, one-half at a time, allowing at least a ½ in. overlap. Each material was tested both as a single and as a double coating. The waterproofing agents were allowed to dry at room temperature without forced draft for approximately the time indicated in Table I. The boxes were then immersed in water and left until failure, or for one month, whichever period was shorter.

Those materials that showed promise in the initial test were subjected to a rough handling test. Four cartons were given a single dip, and four a double dip in the medium. The packages were then placed in a wooden master container capable of holding 32 packages (two layers, each accommodating four by four cartons). The master container was a style two nailed wood box, constructed according to Canadian Packaging Committee Code No. 11.* The test packages were placed along the side of the box, and excess space in the centre was filled with dummy cartons containing sawdust. The loaded master container was stored at −40° F. overnight, followed by subjection to 20 free falls of three feet each (five falls on each long edge). Test packages were then removed from the wooden box, allowed to reach room temperature, and immersed in water.

As a further test of flexibility, the agents used in the rough handling test were subjected to a low temperature bend test. For this trial, strips of 50 lb. kraft (ream weights refer to 500 sheets, 24 × 36 in.) 1 in. by 8 in. were given single and double dips in the waterproofing medium, allowed to dry, stored at the temperature of test (−10° F. and −40° F.) for two hours, bent quickly (less than one second) around a ½ in. diameter rod, and then examined for cracking or flaking.

An accelerated ageing test was also done on these materials. Single and double dipped packages were stored at 140° F. (low humidity, approximately 6%) for one month, and then subjected to water immersion.

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* *Boxes, Wooden, Nailed. Can. Packaging Committee Code 11. Currently available from Forest Products Laboratories, Dept. of Mines and Resources, Ottawa, Canada.*

TABLE I

INITIAL TEST OF WATERPROOFING AGENTS

(Each result based on two packages)

Material	Approximate drying time, hr.	Results of water immersion, time (days) to failure with:	
		Single coat	Double coat
A. Vinylite resin in xylol, methyl ketone, and methyl isobutyl ketone	0.5	<1	<1
B. Vinyl resins in organic solvents containing added pigments and plasticizers	1.5	6	>30
C. Vinyl chloride acetate copolymer, high molecular weight, dissolved in acetone, plasticized	1.0	9	18
D. Vinyl resin emulsion plus solvents, plasticized	1.0	<1	<1
E. Vinyl resin emulsion plus solvents, plasticized	1.0	<1	<1
F. Vinyl and vinylidene resins in acetone and acetate solvents	1.5	>30	>30
G. Cellulose acetate dope	2.5	2	7
H. Cellulose acetate in acetone and other solvents, plasticized	18	2	16
Ia. Nitrocellulose, 21-22% non-volatile, in a solution of esters, alcohols, and hydrocarbons, plasticized	2.0	>30	>30
Ib. Nitrocellulose, 13% non-volatile, in a solution of esters, alcohols, and hydrocarbons, plasticized	2.0	>30	>30
J. Nitrocellulose in a mixture of ester solvents, plasticized	0.25	18	15
K. Styrene resin solution	2.0	<1	<1
L. Chlorinated rubber solution, plasticized	0.25	<1	<1
M. Corn protein – resin solution	1.0	<1	<1
N. Treated wood oil drier combination (leaves no surface film)	1.5	<1	<1
O. Alkyd, phthalic anhydride content 34% (on solid basis), linseed oil modified, petroleum spirit solvent	48	<1	<1
P. Microcrystalline wax: softening point, 150°-155° F.; penetration (100 gm. at 77° F.), 40-45; flexibility good, see Table III	1-2 Min.	<1	6

Since the shortest drying time possible would be desirable in the application of these materials, the drying time of the more promising samples was determined by a blocking test. Strips of 50 lb. kraft, 1 in. by 4 in., were dipped in the waterproofing material and allowed to dry under room conditions for a measured time. At the end of this time, four of these strips were placed on top of each other, and a 1 lb. weight was placed on top of the pile. The samples were stored at 120° F. overnight, then cooled to room temperature, and examined for blocking (sticking together). If blocking did not occur the drying time was reduced until samples just stuck together after this treatment.

Results

Waterproofing materials that left no surface coating on the box, such as *N* (Table I), failed to produce a watertight package because they did not seal the seams and corners of the boxes. Similarly agents that did leave a surface film but had very low viscosity failed to seal the seams because most of the waterproofing compound ran off the box before it had a chance to dry. Materials in this category were *A*, *L*, *O*, and probably *K*. The vinyl resin emulsions (*D*, *E*) were not satisfactory.

On the basis of the initial test for water resistance (Table I), nine of the materials appeared more promising than the rest and were selected for further study. These nine materials are listed in Tables II and III. The rough handling and ageing tests (Table II), indicated that waxes would not be suitable for this purpose if severe handling conditions were encountered. Agents *B*, *F*, *G*, and *H* were also severely affected by these tests. In addition materials *B*, *F*, and *II* all required over 15 hr. to dry (Table III), which further detracted from their value. It is possible that drying times might have been

TABLE II

ROUGH HANDLING AND AGEING TEST OF PACKAGES TREATED WITH WATERPROOFING AGENTS

(Each result based on four packages)

Material	Results of water immersion after rough handling Time (days) to failure with:		Results of water immersion after ageing Time (days) to failure with:	
	Single coat	Double coat	Single coat	Double coat
B	<1	<1	20	>30
C	20	>30	>30	>30
F	2	4	>30	>30
G	<1	10	6	10
H	<1	<1	8	8
Ia	<1	>30	>30	>30
Ib	<1	>30	>30	>30
J	>30	>30	>30	>30
P	<1	<1	<1	<1

TABLE III

FLEXIBILITY AND MINIMUM DRYING TIME OF WATERPROOFING AGENTS

Material	Temperature of bend test				Minimum drying time, hr.
	−10° F.		−40° F.		
	Single coat	Double coat	Single coat	Double coat	
B	Pass	Pass	Pass	Cracked	>15
C	Pass	Pass	Pass	Pass	1.0
F	Pass	Pass	Pass	Pass	>15
G	Pass	Pass	Pass	Pass	0.75
H	Pass	Pass	Pass	Cracked	>15
Ia	Pass	Pass	Pass	Pass	0.75
Ib	Pass	Pass	Pass	Pass	0.75
J	Pass	Pass	Pass	Pass	0.25
P	Pass	Pass	Pass	Cracked and flaked	—

reduced somewhat by using forced air circulation and heating devices, but under these conditions it was difficult to avoid the formation of bubbles in the coating when the solvent evaporated rapidly. The approximate drying time given in Table I does not always agree with that in Table III because the time shown in Table I is the time required for the material to be dry to the touch, whereas Table III gives the drying time required to eliminate blocking. Materials J, C, Ia, and Ib (the last two as double coats) appeared to be superior to the other agents and their relative merit fell in that order. It will be noted that all of these except C were nitrocellulose formulations, and C was a vinyl resin solution. However, it seems likely that some of the other materials tested such as chlorinated rubber, cellulose acetate, and polystyrene could have been improved considerably by changing their composition somewhat, i.e., by better plasticizing, or by increasing the viscosity either by increasing the non-volatile content of the solution or by using more highly polymerized resin.

The results indicate that this method of making waterproof packages would probably work quite satisfactorily using a waterproofing compound such as J, which required only 15 min. to dry under room conditions, and produced packages that withstood water immersion for over 30 days after rough handling at −40° F.

Acknowledgments

DRIED WHOLE EGG POWDER

XXV. FURTHER STUDIES ON THE EFFECT OF ADDED SUBSTANCES

By R. L. Hay, Margaret Reid, and Jesse A. Pearce

DRIED WHOLE EGG POWDER

XXV. FURTHER STUDIES ON THE EFFECT OF ADDED SUBSTANCES[1]

BY R. L. HAY,[2] MARGARET REID,[2] AND JESSE A. PEARCE[2]

Abstract

A number of substances added to liquid egg before drying had no effect on changes in palatability, fluorescence value, and total carotinoid pigment content of the subsequent powders when stored for 16 weeks at 80° F., for eight weeks at 100° F., or for four weeks at 120° F. Added soya lecithin did not increase the initial fluorescence, but egg lecithin did; added lecithin from either source did not accelerate fluorescence changes during storage. Measurement of vitamin A content in treated or untreated powder showed that loss of this component during four weeks' storage at 120° F. was less marked than during 16 weeks' storage at 80° and 100° F., but, at these lower temperatures, heated whole egg powder, heated dried white, heated dried yolk, and heated or unheated cystine or methionine had a preservative action. Sucrose or sodium bicarbonate retarded vitamin A loss in stored dried yolk. Foaming volume measurements on freshly dried powders containing Nacconol S.N.F., Duponol, Roccal, and Aerosol showed that, in general, the addition of these wetting agents to liquid egg before drying adversely affected the aerating power of the powder. The addition of sucrose improved the baking quality of freshly prepared powders, the addition of lactose had a negligible effect and the addition of whey solids reduced the baking quality. When the powders were stored at 80° and 100° F. these three added substances had a preservative effect; sucrose being most effective and whey solids least effective.

Introduction

Dried egg deteriorates during storage even at moisture and temperature levels well below those currently in use by commercial egg drying establishments. As a result, several studies have appeared on the use of added substances in retarding deterioration (1, 9, 12, 15). Previous work in these laboratories showed that sucrose or sodium bicarbonate, when added to whole egg before drying, were effective in retarding loss in quality during subsequent storage (4, 12). Stored egg powder containing lactose has been found to have greater foaming power than a similar powder containing the same concentration of sucrose (2). The addition of 10% of spray-dried whey (containing about 70% lactose) to egg liquid prior to drying had the same protective effect as the addition of 10% lactose when the powders were stored at 117° F. (2).

Other substances likely to affect the keeping qualities of egg powder during drying and storage were indicated by work in related fields. Calcium acid phosphate and phenyl mercuric nitrate offered points of theoretical interest— the phosphate because of its use in baking, and the nitrate because it is believed to react with sulphur-containing compounds. If the sulphur-containing groups were involved in fluorescence development in egg powder,

it was thought that their combination with a mercuric compound might prevent development of fluorescing substances. Lecithin, a component of egg commonly believed to be responsible for the development of off-flavours in many food products, may have been responsible for fluorescence increases in stored egg powder and thus warranted study. Since the previously noted (12) beneficial effect of sodium bicarbonate might be attributable to the presence of the carbonate ion, it was also believed advisable to ascertain whether carbon dioxide added before or during drying would retard fluorescence changes in stored powders.

During preliminary work (11) using a model spray drier (16) it had been observed that excessive heating during the drying operation seemed to prevent loss (oxidation) of vitamin A content in stored egg powder. Since this antioxidant action may have been caused by changes in the sulphur-containing amino acids, added cystine or methionine might prevent excessive loss of vitamin A in stored egg powder.

Reconstituted dried egg is seriously deficient in foaming power. It was believed that wetting agents, if their presence did not accelerate deterioration, might facilitate the reconstitution of egg powder and thus increase the foaming power.

The present paper amplifies some of the foregoing observations by describing: the changes in palatability and fluorescence of stored egg powders containing substances likely to affect deterioration of quality; the changes in vitamin A content of stored dried egg containing substances with possible antioxidant effect; the effect of wetting agents on the foaming power of egg powder; the changes in the baking quality of egg powders containing various amounts of sucrose, lactose, and whey; and the effects of sucrose or sodium bicarbonate on dried yolk or dried white.

Materials and Analytical Methods

All egg powders used in the investigation were prepared in a laboratory spray drier (16) at an inlet temperature of 250° F. and an outlet temperature of 140° F. and were adjusted to final moisture contents between 2 and 3%. Substances under study were added to the liquid egg before drying.

Palatability was assessed by a panel of 14 people, who tasted each sample as scrambled egg. The procedures used for reconstituting the powder and for scoring were the same as those described in an earlier publication (10).

A previously described procedure (10) for measuring fluorescence was used throughout, with the exception that a greater number of defatting operations was necessary to remove all the fat from the dried yolk.

The procedure used for measuring total carotenoid pigments provided a rough arbitrary evaluation of the amounts present in the powder (14). One gram of dried whole egg powder was weighed on an analytical balance to 5 mgm. The sample was transferred to a 250 ml. Erlenmeyer flask and 100 ml. of redistilled acetone was added. The flasks were corked tightly and

the mixture was gently swirled intermittently for 10 min. The mixture was then poured into an 18-cm. No. 12 Whatman folded filter paper and the filtrate collected in a narrow-necked glass vessel, which was tightly corked immediately and kept closed until needed. The transmission of this filtrate was measured, at 444 and 465 mμ on a Lumetron Colorimeter using matched square cuvettes. Redistilled acetone was used as the standard of 100% transmission.

Vitamin A estimation was done by a method outlined by Dann and Evelyn (3). The L values in Table V are for the total blue colour produced by each sample, no correction being applied for any remaining carotinoid pigments.

Foaming volume has been found suitable, with reservations, as a measure of the baking quality of egg powder (4, 7, 13). The foaming volumes of some unstored powders were determined by two procedures: in the first, 40.5 gm. of powder was mixed thoroughly with 75 ml. of distilled water and then beaten for 10 min. in a 'Mixmaster', set at No. 10 speed; the second eliminated the variable egg solids – added substances ratio, unavoidable in the first method, and maintained the egg solids in each determination at 19 gm. The foam was measured in a graduated cylinder. Foaming volumes of the stored powders containing added carbohydrates were measured using the first procedure.

The baking quality of some of the stored egg powders was also evaluated by measuring the volume of sponge cakes prepared by a procedure described in a previous publication (7).

Results

Effects of Added Substances on Changes in Quality and Carotinoid Pigments of Whole Egg Powder

This portion of the study evaluated the effect of materials added to the liquid egg before drying on changes in palatability, fluorescence, total caro-tinoid pigments, and vitamin A content of stored egg powder. The materials added and the concentrations used are given in Tables I and III. Carbon dioxide was added in unknown concentration but was introduced in two ways; in the first, the melange was saturated with the gas shortly before drying; in the second, the carbon dioxide was introduced into the drier during the drying process. The dried products were stored in tin-plate (air as headspace gas) for 16 weeks at 80° F., eight weeks at 100° F., and four weeks at 120° F. The times for this exploratory work were chosen on the basis of work described in earlier papers and permitted examination of the powders at about the point where they became unsuitable for use as an egg dish. Carotinoid and vitamin A determinations were not done on samples stored for eight weeks at 100° F. but were done on samples held for 16 weeks at this temperature.

As shown in Table I, the added substances used caused no significant change in the eating quality of fresh or stored whole egg powders as assessed by either fluorescence or palatability measurements.

The deteriorative changes in dried egg during storage may be due to the reaction of reducing sugar with amino acids (1, 6, 8). Sulphur-containing

TABLE I

ЕFFECT OF VARIOUS ADDED SUBSTANCES ON THE FLUORESCENCE AND PALATABILITY
CHANGES IN STORED WHOLE EGG POWDER

Treatment of liquid whole egg	Fluorescence value				Palatability	
	Initial	80° F., 16 weeks	100° F., 8 weeks	120° F., 4 weeks	Initial	80° F., 16 weeks
Untreated	15 0	47.4	58 0	84 3	8 0	6.6
Liquid egg saturated with CO_2	13.2	40 1	60 1	86 0	8.0	6.6
CO_2 introduced into air in drier	12 0	47 1	65 0	85.0	7.7	6.0
Whole egg powder, 0.1%[1]	13 0	46.1	65 0	88.0	8.1	6.0
Dried white, 0.1%[1]	13.5	44 4	56 0	74.4	8.0	6.3
Dried yolk, 0.1%[1]	12 9	43 1	61.2	82.0	7.9	6 8
Cystine, 0 005%	14 4	46 0	59 2	81 4	8 1	6 6
Cystine, 0.005%[1]	12.1	43.1	55.3	76 0	8.5	6.0
Methionine, 0 005%	14.1	42 3	58 2	78 1	8.5	5 6
Methionine, 0 005%[1]	14 0	42 0	55.0	68 1	8.1	6.0
Nacconal S.N.F., 0 02%	15 1	41.0	59 3	86 1	7.6	7.0
Duponol, 0 02%	14.0	37.4	57 0	79 4	7 5	6.5
Phenyl mercuric nitrate, 0 08%	13.2	42 1	60.3	85.0	--	—
$CaHPO_4$, 0 2%	12 5	47 3	67 3	82 1	8.5	6.9

[1]*Heated for one hour in a vacuum oven at 212° F.*

amino acids may be involved in this reaction as it progresses in egg powder (6), however phenyl mercuric nitrate did not prevent their action nor did the addition of methionine or cystine increase the fluorescence values. This sugar–protein reaction produces some carbon dioxide (6). The beneficial effect of this gas or of the carbonate ion in preserving stored egg powders (9, 12) may, therefore, be explained by the law of mass action. The negligible effect of carbon dioxide in the present experiment is probably due to failure to attain a sufficiently high concentration in the powder.

The addition of lecithin as a possible cause of fluorescence development was given more detailed study. Commercially available egg and soya lecithin were compared by adding them to different lots of liquid egg at levels of 0, 0.01, 0.1, and 1.0%. In addition, some of each of the lecithins was heated for one hour in a vacuum oven at 212° F. before being added to different lots of liquid egg at the 1% level. The powders containing lecithin and a control sample were held at 80° and 100° F. and the fluorescence was determined initially and after one-, two-, three-, four-, and five-week periods. Since it was difficult to attain homogeneity in the liquid, the fluorescence values were more variable than usual. Therefore the data were subjected to an analysis of variance; the results are shown in Table II. As might be expected, both time and temperature had a significant effect on fluorescence development. From the results in Table II the necessary difference for significance* was calculated, and the samples that did not differ were grouped and averaged. The average values for the groups are given in Fig. 1. Neither heated nor

* *5% Level of statistical significance.*

TABLE II

ANALYSIS OF VARIANCE ON FLUORESCENCE DATA FROM A STORAGE EXPERIMENT
ON EGG POWDERS WITH OR WITHOUT ADDED EGG LECITHIN
OR SOYA LECITHIN

Variance attributable to:	Degrees of freedom	Mean square
Samples	8	254**
Temperature	1	4013**
Time	5	1674**
Samples × temperature	8	9
Samples × time	40	13
Temperature × time	5	280**
Residual (Error)	40	11

** *Surpasses 1% level of statistical significance.*

TABLE IIIA

EFFECT OF ADDED SUBSTANCES ON THE TOTAL CAROTINOID PIGMENTS
OF STORED WHOLE EGG POWDER

Treatment of liquid whole egg	Transmission,[1] %							
	Filter 444 mμ				Filter 465 mμ			
	Initial	80° F., 16 weeks	100° F., 16 weeks	120° F., 4 weeks	Initial	80° F., 16 weeks	100° F., 16 weeks	120° F., 4 weeks
Untreated	89 6	90 1	93 0	93 2	89.4	88.6	93.7	94 4
Whole egg powder, 0 1%[2]	84.7	88 3	93 0	89 7	87 0	85 2	91 0	91 5
Dried white, 0.1%[2]	81 1	82 2	86 5	86 0	82 4	81 8	82 2	90 3
Dried yolk, 0 1%[2]	75 3	74 5	82 5	84 4	76.8	79 1	81.1	86 6
Cystine, 0.005%	82.3	81.6	85 2	85 6	81 3	83 1	87 0	90 5
Cystine, 0 005%[2]	73.0	74.6	81 4	82 0	74.5	76 6	82 4	84 7
Methionine, 0 005%	76.0	76.9	81 6	81 5	77.4	77.8	84 3	85.6
Methionine, 0.005%[2]	81 5	74 4	82 2	89 3	82.5	82 3	85 2	90 3

TABLE IIIB

EFFECT OF VARIOUS SUBSTANCES ON THE VITAMIN A CONTENT
OF STORED WHOLE EGG POWDER

Treatment of liquid whole egg	L value[3]			
	Initial	80° F., 16 weeks	100° F., 16 weeks	120° F., 4 weeks
Untreated	20.2	10.6	7.9	22.4
Whole egg powder, 0.1%[2]	17.8	14.1	10.6	14.7
Dried white, 0.1%[2]	22.4	16.7	18.7	17.5
Dried yolk, 0.1%[2]	17.4	16.4	18.7	17.5
Cystine, 0.005%	19.3	14.1	13.8	18.2
Cystine, 0.005%[2]	19.9	16.0	14.9	19.2
Methionine, 0.005%	16.3	18.7	10.6	17.8
Methionine, 0.005%[2]	18.8	13.8	12.0	16.2

[1] *Varies inversely as the carotinoid pigment content.*
[2] *Heated for one hour in a vacuum oven at 212° F.*
[3] *Varies directly as the vitamin A content.*

unheated soya lecithin affected the initial fluorescence value of the powder but unheated egg lecithin caused a slight increase and heated egg lecithin caused a marked increase in the initial fluorescence value. However, the addition of lecithin did not accelerate fluorescence development during storage.
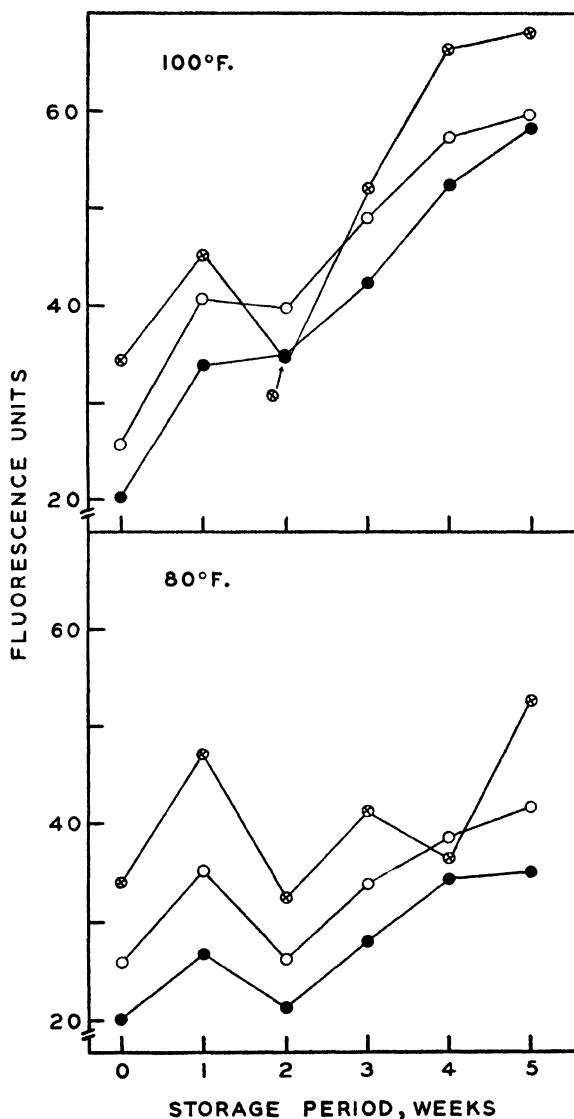
FIG. 1. *The effect of added heated or unheated egg or soya lecithin on the fluorescence changes in stored egg powder.*

● *Control powders and powders containing heated or unheated soya lecithin.*
○ *Powders containing unheated egg lecithin.*
⊕ *Powders containing heated egg lecithin.*

The results in Table III*A*, based on transmission values that vary inversely as the carotinoid content, show that most of the materials, studied primarily for their antioxidant effect, had no significant effect on changes in total carotinoid pigments. This may be partly attributable to wide variation in carotinoid pigments in the freshly prepared powders. Little change occurred during 16 weeks at 80° F. but reduction in carotinoid pigment was noticeable in all samples after 16 weeks at 100° F. and after four weeks at 120° F.

As shown in Table III*B*, all of the addition substances examined in this phase of the investigation (heated whole egg powder, heated dried white, heated dried yolk, and heated or unheated cystine or methionine) had a beneficial effect on vitamin A retention during storage at 80° and 100° F. No significant reduction in vitamin A content was observed in treated or untreated whole egg after four weeks at 120° F. After 16 weeks at 80° or 100° F. the loss of vitamin A was considerable. Previous work showed that loss of vitamin A was pronounced at all storage temperatures from 15° to 100° F. (5).

Effects of Sodium Bicarbonate and Sucrose on Changes in Dried Egg White and Dried Egg Yolk

Further information about the manner in which sucrose or sodium bicarbonate exert a beneficial effect on whole egg was sought by examination of dried white and dried yolk containing these substances. Sodium bicarbonate effectively retarded fluorescence changes in egg white stored at 120° F. and accelerated fluorescence development at 80° and 100° F. (Table IV*A*). This compound was mildly effective in inhibiting fluorescence development in yolk stored at 100° and 120° F. but had no effect at 80° F. Sucrose accelerated deterioration in yolk but had no determinable effect on the white. These and former observations (12) indicate that bicarbonate is most effective in retarding fluorescence development at high storage temperatures. From the data in Table IV*A* and previous work (2, 12) it appears that sucrose is not an effective preservative of eating quality unless used in dried whole egg. These problems are receiving further attention in the laboratories.

As shown in Table IV*B*, the presence of sodium bicarbonate had no effect on total carotinoid pigments in dried yolk. The large quantity of added sucrose diluted the carotinoid pigment content as measured but also appeared to reduce the change during storage. Loss in vitamin A content of yolk was marked under all conditions of study but reduction was retarded noticeably by the presence of sucrose (Table IV*C*).

Effects of Added Wetting Agents on Foaming Volume of Whole Egg Powder

Since wetting agents did not accelerate deterioration, their effect on the foaming power of reconstituted egg was evaluated by adding the following to the melange prior to drying: Nacconol S.N.F., Duponol, Roccal, and Aerosol.

TABLE IV

EFFECT OF SODIUM BICARBONATE AND SUCROSE ON CHANGES IN STORED
DRIED WHITE AND STORED DRIED YOLK

A. Fluorescence value

Treatment of liquid white or yolk	Fluorescence value			
	Initial	80° F., 16 weeks	100° F., 8 weeks	120° F., 4 weeks
White				
Untreated	8 0	41.1	68.0	165.0
Sodium bicarbonate, 0.2%	8.0	66 1	164 0	46.0
Sucrose, 15%	11.7	40.0	86.0	158.0
Yolk				
Untreated	15 0	52.2	57.0	54.0
Sodium bicarbonate, 0 2%	14 0	56.0	48 1	43.0
Sucrose, 15%	10.5	64.0	97.2	165.0

B. Carotinoid content

Treatment of liquid yolk	Transmission, %							
	Filter 444 mμ				Filter 465 mμ			
	Initial	80° F , 16 weeks	100° F., 16 weeks	120° F., 4 weeks	Initial	80° F., 16 weeks	100° F., 16 weeks	120° F., 4 weeks
Untreated	80.1	80 0	86.0	87.0	81 5	81.2	87.1	89.6
Sodium bicarbonate, 0.2%	80.1	82.1	83.5	88.0	81 5	81 0	88.7	90.2
Sucrose, 15%	95.1	95 5	96 8	98 0	95 5	94 5	97.8	99.7

C. Vitamin A content

Treatment of liquid yolk	L value			
	Initial	80° F., 16 weeks	100° F., 16 weeks	120°F., 4 weeks
Untreated	30.7	18.7	18.7	19.2
Sodium bicarbonate, 0.2%	26.2	21.1	14 9	18.7
Sucrose, 15%	22.4	20 3	16.4	21.4

Table V shows that the presence of wetting agents usually had an adverse effect on the foaming volume of plain egg powder. Although powders containing 0.01, 0.02, or 0.04% Duponol had higher foaming volumes than plain

egg powder, similar amounts of added Nacconol, Roccal, and Aerosol had either a negligible or a detrimental effect. For all powders containing higher quantities of wetting agents the foaming volumes were lower than for untreated powders.

TABLE V

EFFECT OF ADDITION OF WETTING AGENTS ON FOAMING VOLUME OF WHOLE EGG POWDER

Concentration of wetting agent, %	Foaming volume, ml.			
	Nacconol	Duponol	Roccal	Aerosol
0	323	323	323	323
0.01	324	356	314	326
0.02	312	368	321	330
0.04	279	349	303	322
0.08	286	264	301	310
0.20	269	298	298	272
0.50	271	258	279	263

Effects of Added Sucrose, Lactose, and Whey on Foaming Volume and Baking Quality

Samples of egg powder containing 0, 10, 20, 25, 30, 35, or 40% sucrose, lactose, or whey solids were prepared. Some samples were examined for foaming power and baking quality before storage while others were sealed in air and stored at 80° and 100° F. Samples were examined by foaming volume only after four weeks' storage at 80° F. and after two weeks' storage at 100° F. At about this time, it became apparent that foaming volume was not a satisfactory measure of baking quality and the final testing, after 12 weeks' storage at 80° F. and eight weeks at 100° F. was for baking volume only.

Two procedures for foaming volume were used since it was difficult to add either carbohydrate or egg in such a way that all components would have identical prior treatment. The results obtained using both foaming volume procedures were similar in nature and are presented graphically in Fig. 2. In subsequent work only the procedure based on a constant amount of total solids in the mix was used.

Fig. 2 shows that the higher quantities of added sucrose resulted in a product with much greater foaming volume than that of untreated powders. Large quantities of lactose had practically no effect and large quantities of whey solids affected foaming volume adversely. It was also noted that powders containing 12% or more whey solids had an offensive odour that might prove harmful to the palatability of baked products.

Results after storage (Fig. 3) showed that, in addition to having the best initial foaming volumes, sucrose treated powders were most stable in this attribute during storage at 80° and 100° F. for four and two weeks respectively. Further examination by baking volume measurements (Fig. 3) also showed the marked beneficial effect of added sucrose on the baking quality of fresh and stored powders. Contrary to the results observed with the foaming
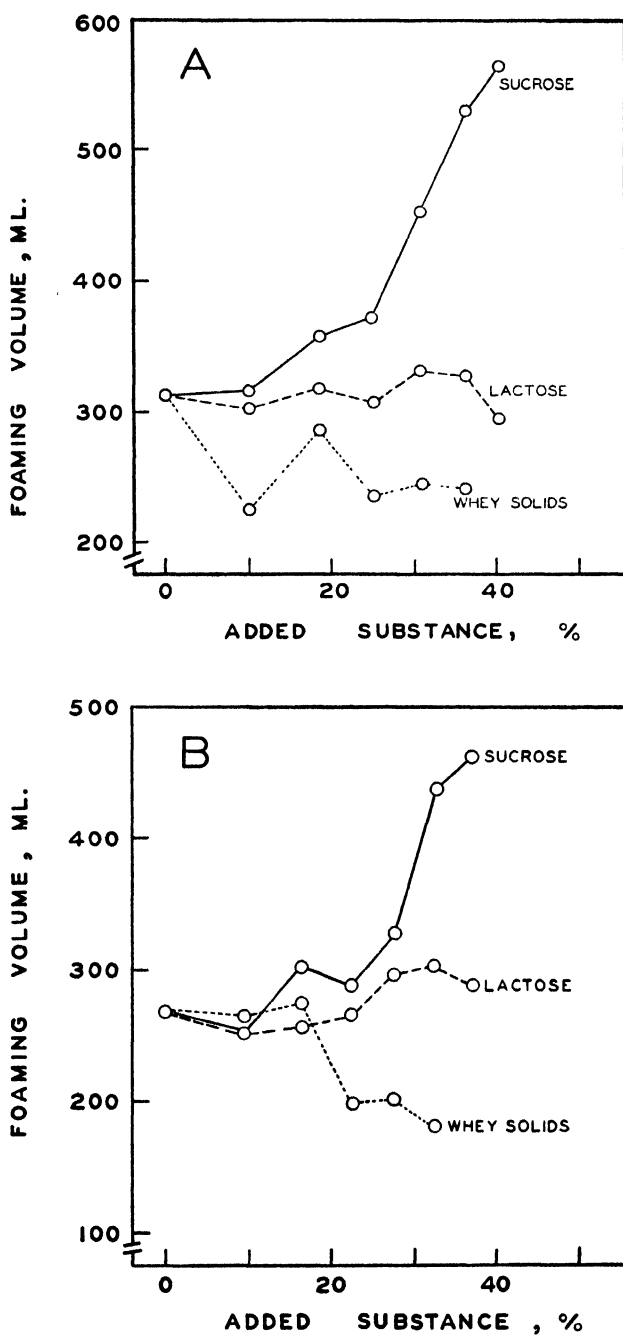
FIG. 2. *The effect of added sucrose, lactose, and whey solids on the foaming volume of egg powder; A, total solids in the mix are kept constant; B, egg solids in the mix are kept constant.*

volumes, lactose improved the baking quality of the fresh powders and provided some protection to stored powders. Added whey had an adverse effect on the baking volume of freshly prepared powder but retarded changes in the stored powders.
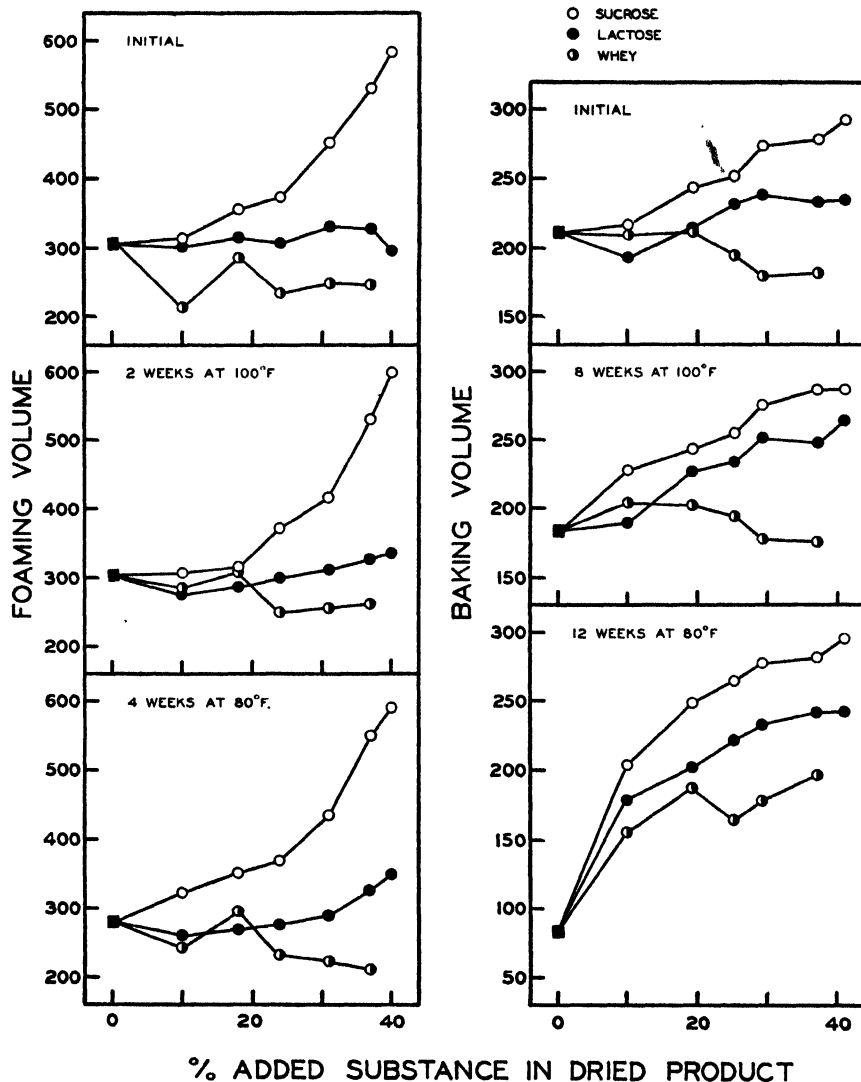
FIG. 3. The effect of added sucrose, lactose, and whey solids on the foaming volume (total solids in the mix are kept constant) and baking quality of stored egg powder.

The marked protective action (in terms of aerating power) provided by sucrose, a non-reducing sugar, and the lesser protective action of lactose, a reducing sugar, when considered in relation to the sugar–protein reaction is

at present unexplainable. This is receiving further attention in these laboratories.

Foaming Volume and Baking Quality of Mixtures of Dried Whole Egg and Dried Whey

To test the effect on foaming volume of whey added to whole egg powder, 0, 10, 20, 30, or 40 gm. of dried British or Canadian whey solids was mixed with 19 gm. of plain egg powder, 56 ml. of water was added, and the mixture was beaten and measured in the usual manner. The resulting foaming volumes, given in Table VIA, indicated that sponge cakes prepared from egg powder with part of the sucrose replaced by whey would be lower in volume than cakes produced from dried egg and sucrose.

TABLE VI

FOAMING VOLUME AND BAKING QUALITY OF MIXTURES OF DRIED
WHOLE EGG AND DRIED WHEY

A. Foaming volume

Amount (gm.) of dried whey added to 19 gm. of whole egg powder	Foaming volume, ml.	
	Canadian dried whey	British dried whey
0	186	180
10	160	148
20	154	114
30	137	104
40	121	101

B. Baking quality

Weight (gm.) of sugar in baking formula replaced by British dried whey	Baking volume, ml.
0	209
1	210
3	182
7	126
15	93

To verify this, 0, 1, 3, 7, or 15 gm. of spray-dried British whey was mixed with 6 gm. of plain egg powder and sucrose was added to make the whey and sucrose for each determination total 20 gm. The solids were mixed thoroughly with 19 ml. of tap water, followed by beating for 10 min. in a 'Mixmaster' set at No. 10 speed. Then 20 gm. of standardized super cake flour was incorporated with the least amount of mixing. The remaining portion of the baking procedure used here was the same as that described previously (7). The volumes of sponge cakes made using these mixtures are given in Table

VI*B* and confirmed the above indication that the volume of sponge cakes prepared by substituting dried whey for sucrose decreased as the amount of added whey increased.

Acknowledgments

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ANTIMONY TRICHLORIDE – ETHANOL PRECIPITATION FOR THE FLUOROMETRIC DETERMINATION OF RIBOFLAVIN IN PORK[1]

By Jessie R. Lewis[2] and Paul R. Gorham[3]

Abstract

A fluorometric method suitable for routine analyses is presented in which interfering substances in papain-takadiastase extracts of pork are precipitated in the presence of 0 02% antimony trichloride and 47 5% ethanol. Antimony trichloride prevents the adsorption of riboflavin upon the precipitate. Recoveries of 95 to 100% are obtained. Determinations by this method correlate well with those obtained microbiologically: for eight samples of fresh pork, four cooked, and four uncooked, $r = 94$; for 20 samples of cured pork, four cooked, and 16 uncooked, $r = 98$.

Introduction

The fluorometric determination of riboflavin in materials of low potency such as pork is made difficult by the relatively high proportion of pigments and other fluorescing substances that accompany riboflavin on extraction. Various techniques have been used, both singly and in combination, for minimizing the effect of these interfering substances: solvent extraction (6, 9, 13, 15, 26), adsorption and elution (1, 2, 3, 4, 10, 11, 12, 16, 21), precipitation (11, 13, 15, 23), permanganate oxidation (1, 2, 3, 5, 10, 11, 12, 16, 17, 18, 21, 24), and stannous chloride reduction (9, 15, 24). Usually, a correction must be applied for the fluorescence of non-riboflavin materials that still remain. Methods for destroying or quenching the fluorescence of riboflavin to obtain this correction factor include photolysis (7, 8, 21), reduction with sodium hyposulphite* (15, 17, 26), and treatment with strong alkali (19).

A proposed study of the effect of various curing methods on the retention of several of the B-complex vitamins in pork muscle required a rapid riboflavin assay that would give results in good agreement with those obtained by slower microbiological methods. The time-consuming adsorption and elution step frequently used for low-potency materials was avoided since complete adsorption of riboflavin from the extract is questionable (3, 16). Extensive trials of several existing methods (15, 19, 22, 23) gave unsatisfactory results. This led to the development of the following procedure, which utilizes a portion of the papain–takadiastase extract used for assaying other members of the B-complex.

* *$Na_2S_2O_4 . 2H_2O$, often sold as sodium hydrosulphite, powdered.*

Procedure

Weigh into a 250 ml. centrifuge tube 5 to 15 gm. of minced sample and add 100 ml. of acetate buffer, pH 4.6, containing 0.4% of a mixture of equal parts of papain and takadiastase. Digest at 37° C. with constant agitation for 16 hr. Filter through Whatman No. 12 paper into a 200 ml. volumetric flask, adjust pH to 6.8 with 4 to 5 ml. N sodium hydroxide, and make up to volume.

Transfer a 25 ml. aliquot of this extract to a 100 ml. centrifuge tube. From automatic burettes add, with constant swirling, 1 ml. of 1% antimony trichloride in absolute ethanol followed by 24 ml. of 95% ethanol. Stopper the tubes, allow 15 min. for precipitation (preferably in the cold), and then centrifuge for five minutes at 2000 r.p.m.
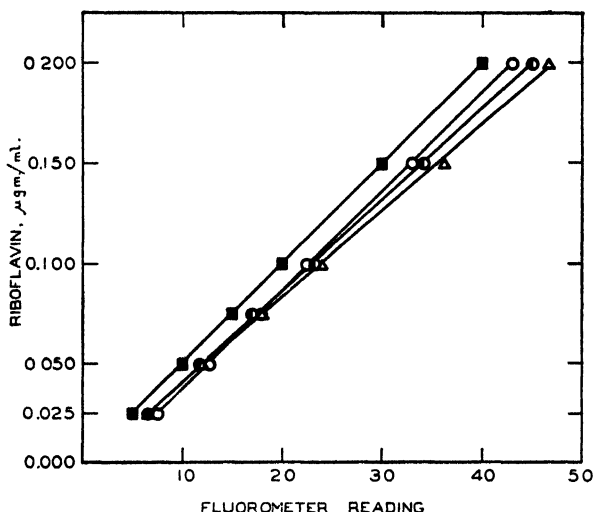
FIG. 1. *Riboflavin reference curves for various solvents. Water or 0.02% antimony trichloride in 47.5% ethanol ■. 47.5% ethanol ○, 23.75% ethanol ◐, 50% acetone △. Each value is the mean of duplicate determinations.*

Take a 10 ml. aliquot of the supernatant liquid and determine its fluorescence with a Coleman No. 12 photofluorometer, using 0.1 μgm. per ml. sodium fluorescein to set the instrument at a reading of 50. Have all samples at room temperature when determining fluorescence. For the blank correction, add 0.25 ml. of a freshly prepared ice-cold solution containing 2 gm. sodium hyposulphite and 0.7 gm. of sodium bicarbonate in 20 ml. of water. Mix thoroughly, determine the residual fluorescence, and subtract this reading from that initially obtained. From an antimony trichloride – ethanol dilution curve (Fig. 1) convert this difference into concentration in micrograms per millilitre and multiply by the dilution factor to obtain the riboflavin content of the sample.

The importance of obtaining the blank correction with as little change in hydrogen ion concentration as possible needs to be emphasized. The use of strong alkali (19) to shift pH to 11 may largely destroy the fluorescence of riboflavin, but may equally well affect the fluorescence of the non-riboflavin materials being assessed. Moreover, with pork extract, it was found to create a turbidity that affected the precision of measurement. The use of 10 to 20 mgm. of solid sodium hyposulphite (4, 17) has been recommended, but addition by means of a solution is more convenient. Sodium hyposulphite readily decomposes in water, producing an acid solution that diminishes the fluorescence of non-riboflavin materials in pork extract. The recommended solution of sodium hyposulphite is stabilized somewhat by the sodium bicarbonate. It has a pH of 6.55, and the addition of 0.25 ml. to 10 ml. of extract at pH 6.8 does not alter the hydrogen ion concentration appreciably.

It should be noted that riboflavin in antimony trichloride – ethanol solution appears to the eye to have its yellow-green fluorescence masked by the blue fluorescence of the solvent. However, the sharp cutout of the filter transmitting fluorescent light, 90% transmission at 5400 Å to 0% at 5100 Å, excludes all measurement of the blue fluorescence (18).

Experimental

Initial attempts were made to clear extracts of cured pork by treatment with acetone or ethanol. Thirty-gram samples from eight different cures were extracted with papain–takadiastase, filtered, and each made to a volume of 200 ml. Aliquots of 25 ml. from six of the extracts were precipitated by an equal volume of acetone, and from four of the extracts by an equal volume of 95% ethanol. The solutions were filtered through Whatman No. 12 paper and the fluorescence of the filtrates was measured. Since riboflavin fluoresces more strongly in acetone or ethanol solution than it does in water (Fig. 1), the measurements were referred to dilution curves for the appropriate solvent in order to calculate the riboflavin content of the samples. Comparative microbiological determinations* were made upon the eight extracts. The results, expressed on a fresh-weight basis, are presented in Table 1. The solvent precipitation and microbiological values were generally in poor agreement.

Significant losses of riboflavin occurred when small amounts of phosphotungstic acid, silver nitrate, copper hydroxide, or zinc hydroxide were added to pork extract treated with ethanol. The addition of 10 to 30 mgm. of antimony trichloride to 50 ml. of a mixture of equal parts of pork extract and 95% ethanol, however, appeared to improve clarification without causing loss of riboflavin.

Six uncooked (15 gm.) and three cooked (10 gm.) samples of cured pork were extracted and each made to a volume of 200 ml. Two aliquots of 20 ml. were taken from each extract. Five millilitres of water containing 5 μgm.

* All microbiological determinations were made by the method of Snell and Strong (25) modified by additions to the basal medium.

TABLE I

COMPARATIVE RIBOFLAVIN ASSAYS OF CURED PORK BY THREE DIFFERENT METHODS,
CALCULATED ON A FRESH-WEIGHT BASIS

| Sample | Acetone precipitation | | | Ethanol precipitation | | | Micro-biological |
	Reading	Blank	μgm./gm.	Reading	Blank	μgm./gm.	μgm./gm.
1	28 5	8.0	1.1	—	—	—	1.6
2	37.0	9.5	1.5	—	—	—	1.9
3	53.5	7.5	2.6	—	—	—	4.0
4	45.0	7.5	2.1	—	—	—	3.2
5	25.5	6.5	1.1	25.5	4.5	1.2	2.0
6	30.0	7.5	1.3	32.0	6.5	1.5	2.5
7	—	—	—	55.0	10.0	2.8	3 7
8 (a)	—	—	—	20.5	1.5	2 2	3 6
8 (b)	—	—	—	19.5	1.5	2.1	3.6

of riboflavin was added to one aliquot and 5 ml. of water was added to the other. All samples were cleared by the addition of 1 ml. of 1% antimony trichloride in absolute ethanol followed by 24 ml. of 95% ethanol, and centrifuging at 2000 r.p.m. for five minutes. An aliquot of the supernatant solution was taken and its fluorescence determined. The amount of riboflavin in 20 ml. of extract was calculated from a reference curve for 0.02% antimony trichloride in 47.5% ethanol (Fig. 1). The fluorometer readings, blank corrections, amounts of riboflavin per aliquot, and percentage recovery are given in Table II. By repeatedly checking the photofluorometer against the fluorescein standard, and making all readings to the nearest 0.25 of a scale division, recoveries of 95 to 100% were obtained.

A somewhat different form of recovery experiment was performed upon an extract of fresh pork to compare the effects of clarification by ethanol, acid

TABLE II

RECOVERY OF RIBOFLAVIN ADDED TO EXTRACTS OF CURED PORK

| Sample | Initial | | | 5 μgm. added | | | Recovery, % |
	Reading	Blank	μgm./20 ml.	Reading	Blank	μgm./20 ml.	
Uncooked:							
1 (a)	15.0	4 0	2.75	35.0	4.0	7.75	100
1 (b)	15.0	4 0	2.75	35.0	4.0	7.75	100
2	9.75	3.0	1.69	29.75	3.0	6 69	100
3	18.25	5.5	3.19	37.5	5.5	8.00	96
4	17.5	5.25	3.06	37.5	5.25	8.06	100
5	16.5	3 5	3.25	35.5	3.5	8.00	95
Cooked:							
5	20.25	5 5	3.69	40.25	5.5	8.69	100
6	15.5	6 0	2.38	35.5	6.0	7.38	100
7	20.25	8.5	2.94	40.25	8.5	7.94	100

ethanol, and antimony trichloride – ethanol. The extract from 90 gm. was adjusted to pH 6.75 and made to a volume of 1200 ml. The following amounts of riboflavin: 0, 1.25, 2.5, 3.75, 5.0, 7.5, and 10.0 μgm., each contained in 5 ml. of water, were added to 20 ml. aliquots of the extract. Twenty-five millilitres of 95% ethanol was added to each, and, after centrifuging, fluorometric readings were made. In a second series, 25 ml. of 95% ethanol made to pH 2.62 with a few drops of concentrated hydrochloric acid was used. A third series employed 1 ml. of 1% antimony trichloride in absolute ethanol and 24 ml. of 95% ethanol (in combination these have a pH of 2.62) for precipitation. The hydrogen ion concentration of each series of clarified extracts was determined. A reference curve for acid ethanol proved to be the same as the curve for water. In Table III, the riboflavin contents of the initial aliquots have been calculated from reference curves for the appropriate solvents. In the same way, increments in fluorescence caused by added

TABLE III

THE EFFECT OF THREE CLARIFICATION TREATMENTS UPON THE RECOVERY OF
RIBOFLAVIN ADDED TO AN EXTRACT OF FRESH PORK

Treatment	Initial			Recovery, μgm./20 ml.						Average pH
	Reading	Blank	μgm./20 ml.	Added riboflavin						
				1 25	2 50	3 75	5 00	7 50	10 00	
Ethanol	15 5	3 5	2 40	1 00	2 25	3 25	4 70	7 10	9 65	6 90
Acid ethanol	15 5	3 0	3 13	1 25	2 50	3 75	5 00	7 50	10 00	6 75
Antimony trichloride – ethanol	15 0	3 0	3 00	1 25	2 50	3 75	5 30	7 50	10 00	6 65

riboflavin have been calculated as recovered riboflavin. Recoveries were lower and the blank was slightly higher with ethanol clarification than with the other two treatments. Ethanol somewhat decreased the hydrogen ion concentration as well.

Since the antimony trichloride – ethanol precipitates appeared more dense, a test was made to determine whether this treatment precipitated more material from the extract than did the acid ethanol or ethanol. This was done by means of nitrogen determinations since the reaction of antimony trichloride with water to precipitate antimony trioxide (20, p. 473) precluded weighing. The precipitates from the foregoing experiment were pooled by treatments. Each combined precipitate was washed twice with 47.5% ethanol, dispersed in water, and made to 50 ml. Duplicate micro-Kjeldahl determinations were run on each as well as on a sample of the unprecipitated extract. The amount of nitrogen precipitated from the enzyme-hydrolysed pork extract was not great. Antimony trichloride – ethanol precipitated 4.0%, acid ethanol 3.4%, and ethanol 3.2% of the total nitrogen in the extract.

The antimony trichloride – ethanol procedure was used for the determination of riboflavin in four samples of fresh and four samples of cooked pork. Sampling and analyses were carried out in duplicate and the results compared with microbiological determinations on the same extracts. The means of duplicate determinations by the two methods were highly correlated $(r = .94)$, as illustrated by Fig. 2A. The standard error of a single determination was
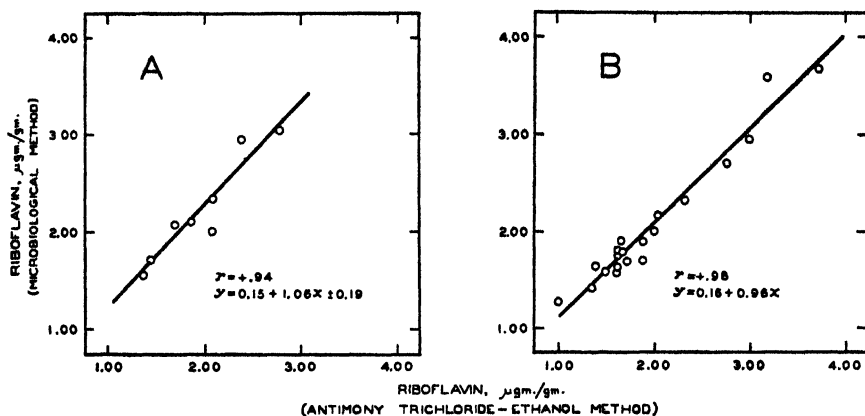
FIG. 2.   *Correlations of riboflavin determinations by the antimony trichloride – ethanol and microbiological methods.   All values calculated on fresh-weight basis.*
    A.   Means of duplicate determinations on fresh pork, uncooked and cooked.
    B.   Single determinations on cured pork, uncooked and cooked.

± 0.16 μgm. per gm. for the fluorometric method and ± 0.08 for the microbiological method. The standard error of prediction of the microbiological value from the fluorometric value was ± 0.19 for the means of the two determinations, and was only slightly greater than the expectation from the variance of duplicates alone. Twenty samples of cured pork, four cooked and 16 uncooked, were extracted and single determinations of riboflavin were made by both microbiological and antimony trichloride – ethanol methods. The correlation coefficient for the determinations by the two methods was .98 (Fig. 2B).

Discussion and Conclusions

Acetone and ethanol both intensify the fluorescence of riboflavin, so that the linear relations with concentration are different from the relation for water or antimony trichloride – ethanol (Fig. 1). Antimony trichloride in contact with water forms hydrochloric acid and a bulky white precipitate, antimony trioxide (20, p. 473). It is this hydrochloric acid that decreases the fluorescence of riboflavin in ethanol for the same reference curve as that for water was obtained when a few drops of concentrated hydrochloric acid were substituted for the trichloride.

Solvent precipitation as a means of removing interfering substances has been noted as untrustworthy (15) because riboflavin may become adsorbed

on the precipitate. The fact that ethanol and acetone precipitation both gave results that were lower than those obtained microbiologically (Table I) tends to confirm this view. With antimony trichloride – ethanol precipitation, however, good agreement with microbiological assays (Fig. 2) and good recoveries of added riboflavin (Table II) were found. Low recoveries over a fairly wide range of concentration occurred when ethanol clarification was tested, but the addition of hydrochloric acid or antimony tricholoride to the ethanol resulted in almost perfect recoveries (Table III). This suggests that the presence of hydrochloric acid released by the antimony trichloride prevents the adsorption of riboflavin upon the precipitate.

It may be inferred from the good recoveries obtained using an external reference curve, that antimony trichloride – ethanol does not alter the fluorescence of riboflavin in pork extract. Therefore, an internal reference standard (6, 19, 22) with its more involved calculation is unnecessary. The external reference curve is so reproducible that routine calculations may be simplified further by multiplying the corrected readings by the factor 0.005 to convert them to concentrations in micrograms per millilitre.

In view of the high degree of correlation between microbiological and antimony trichloride – ethanol results, this method has been adopted by these laboratories for the routine assay of riboflavin in pork products.

Acknowledgment

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DRIED WHOLE EGG POWDER

XXIV. SOME FACTORS AFFECTING COLOUR[1]

By H. Tessier,[2] J. R. Marier,[3] and Jesse A. Pearce[4]

Abstract

A nine filter colour comparator was used to compare egg powders with strips showing the colour of dried egg as received in the United Kingdom. Drying either fresh or frozen eggs did not affect powder colour but Grade B and Grade C eggs gave a product with a more intense yellow colour than Grade A eggs. The area of Canada in which eggs were produced did not affect the colour of the subsequent powder appreciably but powders prepared from eggs produced in May to July, 1945 were a more intense yellow than those from eggs produced in January to April of the same year. Exposure of the liquid egg to light before drying resulted in powders of a duller grey colour than when the liquid was kept in the darkness. Commercial cone-type driers gave powders of a more intense yellow than commercial box-type driers, while powders prepared on the laboratory drier were paler than those prepared on the commercial driers. Coarse powders scattered less of the impinging light than fine powders but the colour quality of the scattered light was unaffected. The addition of a mixture of two dyes, Tartrazine and Sunset Yellow, to liquid egg before drying improved the colour of the resulting powder.

Introduction

Dried whole egg powder shipped from Canada to the United Kingdom was usually considered to be of excellent quality. However, a general complaint was made about the pale yellow colour of the powder and about a pinkish tinge that appeared in some powders. These complaints arise from differences in colour preferences of the people in the two countries. In England, an egg with a dark yellow yolk is usually preferred, while Canadians usually like an egg with a pale yolk. This and Canadian economics have led to the use of diets for Canadian egg layers that will produce a pale yolk, hence the undesirably pale colour of Canadian egg powder.

Although work in these laboratories (4) and elsewhere (2) had described the darkening that occurred in egg powder during storage, there was little information about other factors that may affect powder colour. Some of these, examined in the present study, are: the type of drier, the time of year and area of the country in which the eggs were produced, the quality of the eggs, and exposure of the liquid egg to light. In addition, powders containing added colouring were examined.

The present paper describes colour measurements on variously treated powders using a nine filter comparator designed in these laboratories (5). Description of the results was facilitated by referring the measurements to a

set of coloured strips depicting the colour of egg powders received in the
United Kingdom and kindly supplied by the Low Temperature Research
Station at Cambridge, England.

Materials and Methods

Since the studies described here were diverse in nature, descriptions of the
materials used will be given in the various subsections of the results.

Both the brightness or intensity and the chroma or colour quality of the
samples were measured by a nine colour method used earlier for meat (5) and
eggs (4).   By this method, colour quality was determined as the light scattered
by the sample within any one of nine colour bands (5).   The light scattered
in any one band is expressed as a fraction of the total light scattered by the
sample in relation to the light scattered by the standard white surface of
magnesium carbonate.   The brightness of the sample was determined as an
item separate from the analysis of colour quality.

Wherever possible, the colour data were treated by statistical methods, and
the values for factors having no significant differences were grouped, averaged,
compared with colour data for the Low Temperature Research Station
(L.T.R.S.) coloured strips, and are recorded here in terms of these strips.

Results

Colour Analysis of Low Temperature Research Station's Coloured Strips

The slides submitted were divided into four groups or series: P_1 to P_3
represented pale, pinkish-coloured egg powders characteristic of some of the
powder produced in Canada; $2Y_1$ to $2Y_5$ showed shades of yellow, typical of
the normal range of colour in American samples; Y_1 to Y_5 resembled the $2Y$
series, but had a greyish-yellow appearance, and represented egg powders that
had lost their lustre as a result of overheating during the drying process or
because of poor storage conditions; and the slides in the B group extended
the Y series and showed badly burnt powders.   In each group the members
with lower numbers were of a paler shade than those with higher numbers.

The results of brightness measurements on the foregoing strips are given in
detail in Table I.   These data show that the P series scattered the greatest
amount of the impinging light, i.e. they were the brightest, and the B series
scattered the least.   The greyish Y series scattered less light than the pink P
group but slightly more than the normally coloured $2Y$ slides.   Within the
various series, the members with higher numbers scattered less light than the
members with lower numbers.

The results of colour quality measurements on the various slides are also
given in detail in Table I.   However, since these detailed data are difficult to
visualize, the colour quality profiles showing the averages of values for each
group are presented in Fig. 1.   These profiles show that the pinkish P series
scattered more light in the blue and blue-green regions of the spectrum (385
to 525 mμ) than any of the other series and that the normally coloured $2Y$

TABLE I

COLOUR ANALYSES OF LOW TEMPERATURE RESEARCH STATION'S COLOURED STRIPS DEPICTING
EGG POWDERS AS RECEIVED IN THE UNITED KINGDOM

Strip number	Bright-ness, %	Colour quality, % scatter								
		Blue			Green			Red		
		385-434 mμ	434-458 mμ	458-487 mμ	487-525 mμ	525-556 mμ	556-584 mμ	584-614 mμ	614-644 mμ	Above 644 mμ
P_1	66 2	4 8	10 5	11 5	18 7	13 2	21 3	9 6	5 7	5 7
P_2	62 4	4 9	10 7	11.4	17.4	13 2	21 2	9 7	5 8	5 7
P_3	62.0	5.0	10.5	11.5	17 3	13 0	20 9	9 9	6 0	5 9
$2Y_1$	56 6	4 8	7 5	9 4	16 1	14 4	24 8	10 7	6 2	6 1
$2Y_2$	56.5	4 4	7 2	9 4	15 6	14 5	25 4	10 9	6 4	6 2
$2Y_3$	51.4	3 9	6 6	9 7	14 5	14 3	26 3	11 4	6 7	6.6
$2Y_4$	49 7	3 8	6.0	9 0	14 3	14 3	27 0	11 8	6 9	6 9
$2Y_5$	45 0	3 1	5 9	9 0	14 1	14 2	27 5	12 1	7 1	7 0
Y_1	59 4	3 9	8 3	10.5	17 2	13.8	23 5	10 3	6 2	6 3
Y_2	59.2	3 9	8 4	10 6	16 8	14 1	23 5	10 3	6 2	6 2
Y_3	56 2	3 7	8 0	10 3	16 5	13 8	23 9	10.7	6 4	6 7
Y_4	52 2	3 8	7.7	10 1	15.7	13 8	24 0	11 0	6 7	7 2
Y_5	51 4	3.5	7.5	10.4	15 5	13 3	24 0	11 3	7.0	7 5
B_1	43 6	2 8	7 6	9 9	15 2	13 4	24 1	11 6	7 2	8 2
B_2	43 4	3 2	7.5	10 4	15 6	13 1	23 7	11 4	7 1	8 0

group scattered more light in the yellow region of the spectrum (525 to 584 mμ). The greyish-yellow Y series showed a slight increase in scatter in the red region (above 644 mμ) and the burnt B series showed a marked increase in this region. The foregoing differences were generally accentuated by changes within the series, from the palest to the most intense shades (Table I).

These preliminary measurements provided a quantitative basis for the examination of some of the factors believed to be responsible for the pinkish colour of some Canadian egg powder. However, measurements necessitated the inclusion of a hypothetical coloured strip, which, from the values reported here, would be lettered $2Y_0$.

The Effect of Particle Size on Egg Powder Colour

Differences in colour were observed for powders prepared in commercial driers and in the laboratory drier (6) and these were attributed in part to differences in particle size. To clarify this point, eight samples of commercially produced egg powders were screened for one hour on a Ro-Tap rotary sifter using sieves of 35, 65, 80, 100, 120, 200, and 325 mesh (U.S. Bureau of Standards) and each fraction of the samples was examined for colour. The average colour profile for all samples and all mesh sizes matched the profile of the yellow $2Y$ series and in particular the $2Y_1$ strip. The major difference was in brightness: the coarse fractions scattered less light than the fine fractions (Table IIA).
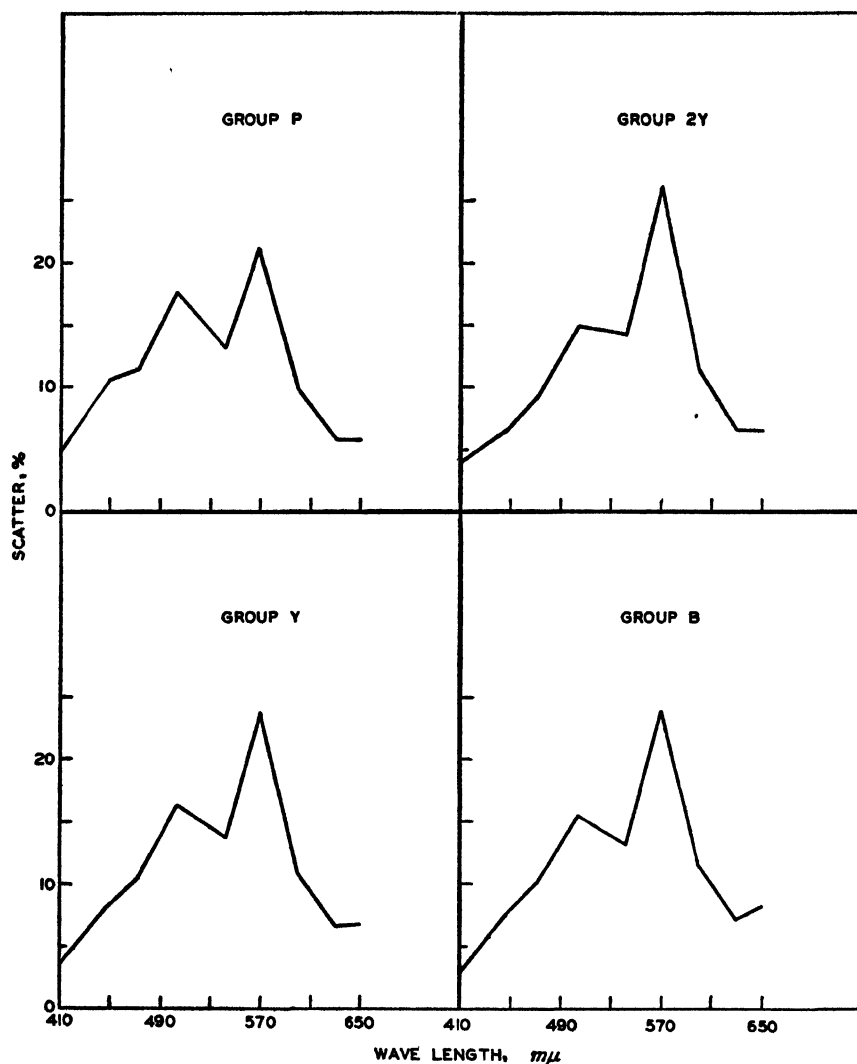
FIG. 1.   *Colour quality profiles (averages) for the groups of coloured strips supplied by the Low Temperature Research Station, Cambridge, England.*

Sieving does not effect a complete separation of the particles of different sizes that are present in egg powder.   Groups of particles may be seen in Fig. 2e at i, ii, and iii.   These groups of particles do not break up during the sieving operation.

Further consideration was given to particle size and colour by the examination of powders prepared in a commercial cone-type drier using spray nozzles of different sizes: 0.065, 0.068, and 0.070 in. in diameter.   The resulting powders matched the $2Y_1$ strip in colour distribution and, again, the finest powder (prepared using 0.065 in. nozzle) was the brightest (Table IIB).

If the particle structure of egg powders is considered, explanation of the foregoing becomes apparent. Fig. 2 shows the microscopic structure of a number of samples. The particles passing a 35 mesh screen and retained by

TABLE II*A*

THE EFFECT OF PARTICLE FRACTIONATION BY SIEVING
ON THE BRIGHTNESS OF EGG POWDERS

Particle range, U.S. Bureau Standards sizes	Brightness, % (average, eight samples)
35–65	49.1
65–80	52.1
80–100	51.8
100–200	53.3
200–325	55.9

TABLE II*B*

THE EFFECT OF SPRAY NOZZLE SIZE ON THE BRIGHTNESS AND
PARTICLE SIZE OF EGG POWDERS PREPARED IN A
CONE-TYPE COMMERCIAL DRIER

Nozzle diameter, in.	Brightness, %	Average particle size, mm.
0 065	56.0	0.03
0 068	54.2	0 05
0.070	51.5	0 4[1]

[1] *Size of clusters. Individual particles could not be measured.*

a 65 mesh screen are given for reference (Fig. 2*a*). These relatively large particles usually exude egg fat, which appears as smears (Fig. 2*a*) or discrete droplets (Fig. 2*f*). Powder from the main chamber usually consists of coarser particles than powder from the secondary chambers (Figs. 2*b*, 2*c*) and the difference between the main chamber and secondary chamber powder from cone-type driers is usually greater than the difference between the corresponding powders from the box-type driers.

The brightness differences recorded in Table II*B* are explained by Fig. 2 (*d*, *e*, *f*). Light is scattered from large particles in many directions and a relatively small proportion is reflected back to the light receptor (human or electric eye), while a powder made up of fine particles presents almost a plane surface and a greater amount of light is reflected in any one direction. From these considerations, and the data in Table I, fine powders should be paler in colour than coarse. The differences attributable to particle size would tend to weaken the comparison between brightness of powders and the brightness of the coloured strips. Nevertheless, it is believed necessary to give consideration to intensity measurements when transforming colour data to terms of the coloured strips.

Comparison of Canadian Egg Powder with L.T.R.S. Strips

It was thought desirable to determine how closely egg powder samples (commercial) could be matched with the various coloured strips by visual inspection, and opportunity was taken to determine whether the pinkish colour was attributable to the use of frozen liquid egg in the production of powder. As shown by the data in Table III, grading by visual examination gave a fairly good approximation of the results obtained by colour analysis. In addition, these results indicated that the use of frozen egg was not the sole cause of the pinkish powders.

TABLE III

COMPARISON OF METHODS OF CLASSIFYING EGG POWDER ACCORDING TO
L.T.R.S. COLOURED STRIPS AND OF THE EFFECT OF THE PHYSICAL
STATE OF THE EGG ON THE POWDER PRODUCED

Physical state of egg used to produce various powders	By colour analysis	By visual inspection
Shell eggs	$2Y_3$	$2Y_2$
Shell eggs	$2Y_0$[1]	P_1
Shell eggs	$2Y_2$	$2Y_3$
Shell eggs	$2Y_2$	$2Y_2$
Shell eggs	$2Y_2$	$2Y_1$
Mixed shell and frozen	P_3	P_2
Frozen melange	P_3	P_1
Frozen melange	P_3	P_3
Frozen melange	$2Y_3$	$2Y_2$
Frozen melange	$2Y_2$	$2Y_2$

[1] *This sample had the characteristics of the yellow 2Y series, but the values matched those of a hypothetical strip in this series that would be numbered $2Y_0$.*

Colour Characteristics of Powders Prepared in Canadian Plants During Various Periods of the Year

Samples of powder were taken from both the main drying chambers and from the secondary collectors of various Canadian egg-drying plants at monthly intervals during the months of June to November, 1943 and in March, 1944. At that time, it was possible to obtain powder from five cone-type driers and from four box-type driers. Two of the box-type driers were rectangular in shape with flat bottoms and the other two had V-shaped bottoms. In general, fresh or stored shell eggs were processed during June, July, and August, frozen melange during September, October, and November and fresh shell eggs in March, 1944.

The results (Table IVA) showed that on the average all powders collected from the main chambers of the various driers corresponded to the yellow $2Y$ group of slides and that the powders from the cone-type and V-bottom, box-type driers were more intensely coloured than the powders from the flat-bottom box. The powders from the secondary collectors of the cone-type drier were burnt more than the powders from the secondary collectors of the

PLATE I
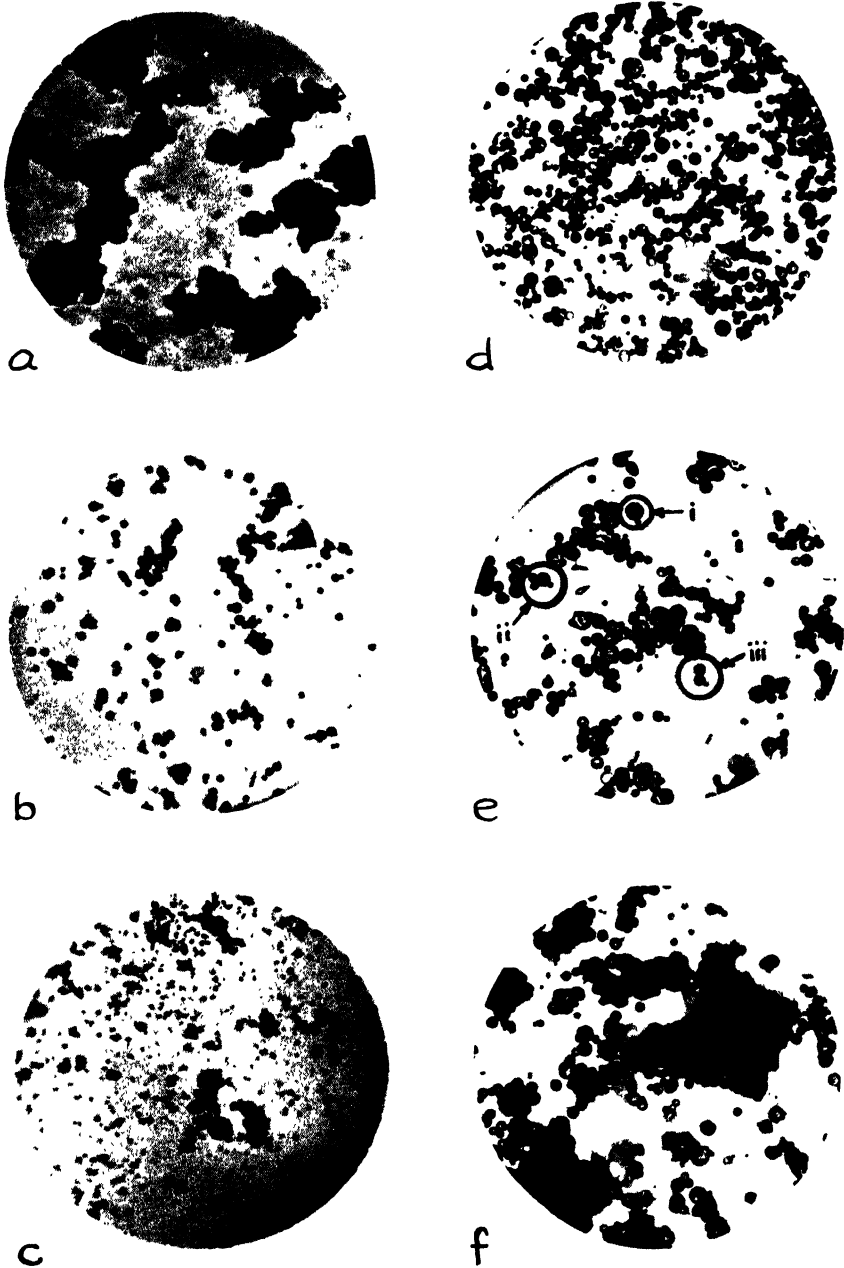
FIG. 2.   *Photomicrographs of egg powder (× 37).*
   (a). Fraction of a powder passing a 35 mesh screen and retained by a 65 mesh screen (U.S. Bureau of Standards).
   (b). Powder from the main collector of a flat-bottomed, box-type drier
   (c). Powder from the secondary collector of a flat-bottomed, box-type drier.
   (d), (e), and (f).   Powders from the main collector of a cone-type drier, prepared using nozzles 0 065, 0 068, and 0 070 in. in diameter, respectively.

TABLE IVA

EFFECT OF DRIER TYPE AND COLLECTION CHAMBER ON THE COLOUR OF
COMMERCIALLY PREPARED EGG POWDER

Type of driers	Number in operation	Grade according to L.T.R.S. coloured strips	
		Main chamber	Secondary collectors
Box, flat-bottom	2	$2Y_1$	Y_1
Box, V-bottom	2	$2Y_3$	Y_4
Cone	5	$2Y_4$	B_1

other two types. Since the powder caught in the secondary collector is finer than the powder trapped in the main chambers, it is reasonable to expect that these particles had dried faster and hence were more likely to be burnt.

Only the powder prepared in March, 1944 differed from the other powders (Table IVB). This supported previous evidence that the use of frozen egg had little effect on the colour of powder, but indicated that the time of year during which eggs are produced may have a pronounced effect.

TABLE IVB

EFFECT OF MONTH OF PRODUCTION ON THE COLOUR OF
COMMERCIALLY PREPARED EGG POWDER COL-
LECTED FROM THE MAIN DRYING CHAM-
BERS OF NINE DRIERS

Month of production	Grade according to L.T.R.S. coloured strips
June to November, 1943	$2Y_3$
March, 1944	Y_3

Colour of Powders Prepared in a Laboratory Drier and in Commercial Driers

The survey of powders prepared in various commercial plants indicated that season of production of the eggs may be an important factor affecting the colour of egg powder. Information about this point could best be obtained by using the laboratory spray drier (6); therefore, a comparison was made of the differences in the colour of powder prepared in the laboratory drier and in commercial driers. For this purpose, two lots of frozen melange were used: a portion of one lot processed in western Canada was dried in a V-bottom, box-type drier and another portion in the laboratory drier; and a portion of the second lot processed in eastern Canada was dried in a cone-type drier and a portion in the laboratory drier.

As seen in Table V, the laboratory drier gave normally coloured powders (2Y series) but these powders were a paler yellow than the powders from the commercial driers. This may have been a function of the scale of the laboratory and commercial operations: egg is dried in the laboratory drier at

lower temperatures than in commercial operations. In addition, this study indicated that the region of the country in which the eggs were produced might also affect the colour.

TABLE V

COLOUR OF EGG POWDERS PREPARED IN COMMERCIAL DRIERS
AND IN A LABORATORY DRIER

Source of frozen melange	Type of drier	Grade according to L.T.R.S. coloured strips
Western Canada	Laboratory Commercial, box-type, V-bottom	$2Y_0^1$ $2Y_1$
Eastern Canada	Laboratory Commercial, cone-type	$2Y_1$ $2Y_2$

¹ *See Table III.*

The Effect of Grade of Eggs and Locality and Month of Production on the Colour of the Resulting Powder

A comprehensive study was made of the effects of grade of eggs and the month and locality of egg production on the quality of the powder produced and these results have been reported (3). Colour measurements, made on the same material, are presented here. In brief, the experimental design required shipments of Grade A^* (large, medium, and pullet), Grade B, and Grade C eggs from Vancouver, Edmonton, Winnipeg, London, Belleville, and Three Rivers on the 15th of each month from December, 1944 to July, 1945. Upon receipt in the laboratory the various samples of eggs were dried in the laboratory drier, and therefore, all the colours recorded here are somewhat paler than would be obtained in a commercial drier.

The area of production had no significant effect on the colour of powder prepared from Grade A eggs; the averages of the colour measurements showed that eggs from all areas corresponded to the hypothetical colour strip $2Y_0$, i.e. a very pale powder of a normal yellow shade. It was not possible to get Grade B eggs from the Vancouver area, but a comparison for the other five areas showed that Grade B eggs from Edmonton, Winnipeg, and London gave powders corresponding to the yellow $2Y_2$ strip while a similar grade of eggs from Belleville and Three Rivers gave powders corresponding to the paler $2Y_1$ strip. In general, powders from Grade B eggs were a more intense yellow than powders from Grade A eggs. Unfortunately, insufficient Grade C eggs were received to make a valid comparison of these and Grades A and B eggs for the various areas.

When considering the effect of month of egg production, a marked difference was observed between powders prepared from eggs produced between January and April and those produced between May and July. The data for this

* *Descriptions of the various grades of eggs can be found in (1).*

period were, therefore, subjected to further mathematical analysis (Grade C eggs were included in these considerations). The results in Table VI show that eggs produced in the early part of the year, while still of a yellow colour, were much paler than eggs produced later in the year and Grade C eggs gave a more intensely coloured powder than Grade B eggs, which, in turn, were a more intense yellow than Grade A eggs.

TABLE VI

COLOUR OF POWDERS PREPARED FROM EGGS OF DIFFERENT QUALITY PRODUCED DURING JANUARY TO APRIL AND MAY TO JULY, 1946

Grade of eggs	Period of production	Grade according to L.T.R.S. coloured strips
A	Jan. to Apr.	$2Y_0$[1]
	May to July	$2Y_1$
B	Jan. to Apr.	$2Y_2$
	May to July	$2Y_4$
C	Jan. to Apr.	$2Y_2$
	May to July	$2Y_6$

[1] *See Table III.*

The differences attributable to grades of eggs can be explained on the basis of the Canadian grading system (1). When candled, the yolks of Grade A eggs must be barely discernible, the yolks of Grade B eggs can be somewhat darker, and the yolks of Grade C eggs can be distinctly visible and even mottled. The seasonal differences are probably a reflection of the onset of warm weather and the corresponding transfer of hens from buildings to out-of-doors.

Effect of Sunlight and Ultra-violet Light on the Colour of Egg Powder

The drying described in the previous section was usually done in a fairly well lighted laboratory. During the work, it was observed that samples prepared on a cloudy day were slightly different from samples prepared on a sunny day. Therefore, an examination was made of powders prepared from liquid egg exposed to sunlight or to an ultra-violet light (carbon arc) or from liquid held in total darkness. Powder prepared from liquid exposed to the ultra-violet light had the greyish tinge common to strips in the Y series and corresponded to strip Y_3 while powder from liquid exposed to sunlight corresponded to the normal yellow strips and matched $2Y_3$. Powder from liquid egg held in total darkness was unlike any of the L.T.R.S. strips. The colour quality of this powder when compared with the colour quality of the $2Y$ group (Fig. 1) showed greater scatter in the yellow region of the spectrum (556 to 584 mμ) and reduced scatter in the blue–green region (487 to 525 mμ).

This powder should represent a third group of strips of a more brilliant yellow than any forwarded from England and should be represented by a series called 3 Y.

The Effect of Added Colouring Matter on the Colour of Egg Powder

Since alteration of the foregoing factors would effect little improvement in the colour of egg powder, it was thought possible that a more desirable looking dried egg powder might be prepared by the addition of various colouring materials. For this purpose, the dye combinations listed in Table VII were added in various amounts to liquid egg before drying in the laboratory spray-drier. Of these, none gave a powder with a colour profile similar to any of

TABLE VII

Dyes[1]	Proportions	Amounts, ml., added to one litre of liquid egg
Cheese colouring[2]	—	0.5, 1, 2, 4
Lemon pie colouring[3]	—	5, 10, 20
Tartrazine[4]	—	5, 10, 20
Tartrazine : Ponceau[4]	3 : 1	10
Tartrazine : Sunset Yellow[4]	3 : 1	10
Tartrazine : Sunset Yellow	7 : 1	5, 10
Tartrazine : Sunset Yellow	9 : 1	5

[1] *All but cheese colouring consisted of 5 gm. solid made up to 100 ml. with water: cheese colouring concentration was unknown.*
[2] *Kindly supplied by Chateau Cheese Co., Ottawa, Ont.*
[3] *Kindly supplied by Canadian Doughnut Co., Trenton, Ont.*
[4] *Kindly supplied by Dye and Chemical Co. of Canada, Ltd., Kingston, Ont.*

the series shown in Fig. 1, and many powders were so badly coloured that they were undesirable. Colours approximating the yellow 2 Y series, by visual inspection, were obtained by the use of: 4 ml. of cheese colouring; 10 ml. of pie colouring (5% solution); or 5 ml. of a mixture of 9 parts Tartrazine and 1 part Sunset Yellow (5% solution) per litre of liquid egg. Considerations of cost and ease of handling led to the selection of the Tartrazine – Sunset Yellow mixture. By colour analysis, powders containing this dye scattered less light in the blue region of the spectrum (385 to 487 $m\mu$), and more in the blue–green (487 to 525 $m\mu$) and in the red (584 to 644 $m\mu$) regions than the desirable yellow 2 Y series. However, these powders met the visual requirements and commercial scale, trial shipments to the United Kingdom had a satisfactory colour.

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DRIED WHOLE EGG POWDER

XXVI. SOME OBSERVATIONS ON THE QUALITY OF POWDER PREPARED FROM FROZEN, LIQUID EGG[1]

By Jesse A. Pearce,[2] H. Tessier,[3] C. G. Lavers,[4] and M. W. Thistle[2]

Abstract

Liquid from eggs of various qualities was frozen at $-40°$ F. and stored at $10°$, $0°$, and $-10°$ F. for 12 months. Powder produced from the defrosted samples was assessed by fluorescence and potassium chloride solution solubility measurements, which showed that liquid from musty or incubator reject eggs gave a less desirable powder than liquid from Grade *A*, Grade *C*, or cracked eggs; increase in time of frozen storage decreased the quality of the resulting powder; if frozen storage extended beyond six months, the lowest storage temperature was most desirable; and method of packaging (in Reynold's Metal A-10, or in wax paper with or without added ice) had no effect on the quality of the powder produced. If drying conditions were held constant, increased dilution of the defrosted egg before drying resulted in a poorer powder. It was shown that freezing or defrosting operations should be completed within about one hour.

Introduction

At the time these studies were planned, it was apparent that large quantities of the eggs produced in the spring and summer in Canada and used for subsequent drying would have to be stored. The capacity of the drying equipment available was not sufficient to process during the flush season the total amount of powder required for export, nor even to process the eggs within the time they might reasonably be stored in the shell. Eggs in the shell should be held for no longer than about three months and conditions required that they be held for six to eight months. This long term holding is usually done by breaking the eggs, mixing the white and yolk, and freezing the resulting liquid. However, little information was available to describe the effects of frozen storage of liquid egg on the quality of the resulting powder. This paper describes the results of quality measurements on powder produced from frozen liquid egg.

Materials and Methods

The material used in the preliminary study was prepared from the meats of Grade *A* large, fresh eggs. For this study, 700 ml. aliquots of egg were frozen into blocks 5 × 7 × 1.5 in. at $-40°$ F. The blocks were lashed together in sets of four, separated by wooden strips so that each block had an equal area of surface exposed. The sets were stored unwrapped for 18 months at $0°$ and $15°$ F.: some exposed to the air in the storage rooms; others in closed

desiccators containing 50% and 20% sulphuric acid. Initially, the acid solutions should have given relative humidities of 35% and 87%, but these values no doubt changed slightly until equilibrium was reached.

The later more comprehensive storage study utilized material described in earlier papers (2, 5). In brief, liquid from fresh Grade *A* eggs, from Grade *A* eggs held for 16 hr. at 80° F. in sterile glass containers, from Grade *C* eggs, from cracked eggs, from musty eggs, and from 'eight-day' incubator reject eggs was poured into moulds containing about four litres, frozen within 16 hr. in a room operating at −40° F., and held at temperatures of 10° (+ 2°, − ½°), 0° (± 1°) and −10° (± 1°) F. for a period of one year. Samples were dried before and after freezing and after 3, 6, and 12 months' storage. The liquid from fresh Grade *A* eggs was packed in Reynold's Metal A-10, a highly moisture vapour resistant material (8), plus a Fourdrinier Kraft, B-Flute carton; in wax paper (40 lb. Kraft, waxed to 50 lb.) plus the carton, with ice cubes inside the carton and surrounding the wrapped egg; and in wax paper and carton without added ice. All the other samples were packed in Reynold's Metal A-10 and cartons.

Accessory studies on material prepared from the meats of Grade *A* large, fresh eggs included evaluation of the effects of dilution of the defrosted egg, freezing rates, and defrosting rates. The freezing time was considered as the time for the centre of the block to reach a temperature of −20° F. At this temperature approximately 7% of the melange is still unfrozen (6).

The egg was dried in a laboratory spray drier (10), and for all but the freshly frozen material, the inlet and outlet temperatures were 250° and 140° F. respectively. Dilution of defrosted egg is usually desirable if the viscosity of the material is to be reduced to a point where the egg can be handled by the pump on the drier and was a necessity when using the laboratory drier. In an attempt to keep the dilution constant, the defrosted egg used in the storage study was dried after addition of an equal volume of water. This effected an excessive reduction in the viscosity of freshly frozen egg and, to obtain a dry powder, less material could be put through the drier with a corresponding increase in outlet temperatures (150° to 155° F.). However, it was possible from previously available data, some of which have been published (9), to correct the values for the analytical measurements on these samples to values that would be expected at 140° F., thus bringing all measurements to a reasonably comparable basis.

The quality of the powder was assessed by measurements of fluorescence and potassium chloride solution solubility values (4, 7).

Results

The results of the preliminary experiment are given in Table I. When thawed, most of the samples were viscous and had to be diluted before they would flow through the pump and the drier nozzle: the proportions of added water are shown in the table. It appeared from the analytical measurements

TABLE I

EFFECT OF TEMPERATURE AND HUMIDITY ON FROZEN EGG STORED 18 MONTHS BEFORE DRYING

(Drying conditions: inlet temp. 250° F.; outlet temp. 140° F.)

Storage conditions		Dilution necessary for drying, egg : water	Quality of resulting powder	
Temp., ° F.	Relative humidity, %		Fluorescence value	Potassium chloride value
0	Room	1 : 1	25.6	57
	35	2 : 1	19.5	62
	87	2 : 1	19.6	62
15	Room	1 : 1	25 2	65
	35	2 : 1	24.6	61
	87	Undiluted	21.7	63
Unfrozen egg		Undiluted	18.0	73

that prolonged frozen storage had an adverse effect on solubility as assessed by potassium chloride values, and on eating quality as assessed by fluorescence measurements. There was some evidence that a storage temperature of 0° F. was preferable to 15° F. Greatest deterioration seemed to occur in the samples exposed to the room air, but this may have been attributable to the large quantity of water added before drying.

Since dilution may have affected powder quality, it seemed desirable to consider this factor before proceeding to the next phase of the investigation. The results of drying defrosted egg mixed with different quantities of water (Table II) indicated that dilution does affect fluorescence values adversely, but some dilution was necessary if solubility was to be retained. Diluting

TABLE II

EFFECT OF DILUTION OF THAWED FROZEN EGG ON THE
QUALITY OF POWDER PRODUCED

(Drying conditions: inlet temp. 250° F.; outlet temp. 140° F.
Egg defrosted at 40° F.; time, 48 hr.)

Dilution, egg : water	Fluorescence value	Potassium chloride value
Unfrozen egg		
No dilution	18.9	83 2
5 : 1	21.7	80.0
1 : 1	22 3	78 8
Freshly frozen egg		
No dilution	18 0	67 1
20 : 1	19 0	78.4
7 : 1	26 6	75 4
4 : 1	25 3	73.6
1 : 1	28.5	71.7

fresh liquid egg had only a slight effect on the quality of the subsequent powder. The adverse effects can be partially eliminated if the pump rate is kept constant and the outlet temperature is allowed to vary according to the amount of water present (1). After these trials, it was believed that, to obtain comparable results in subsequent experimental work, the defrosted egg should be diluted with the same proportion of water.

STORAGE, MONTHS
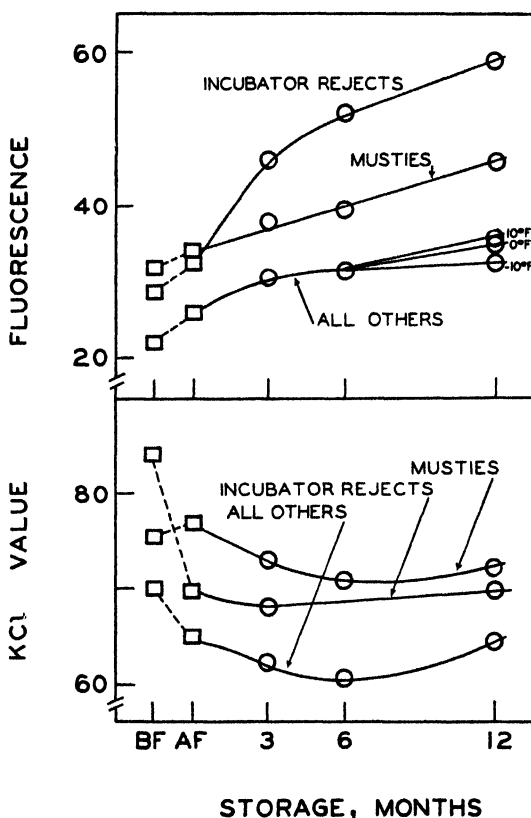
FIG. 1. *The effect of freezing and frozen storage of liquid egg on the quality of powder produced.*
    Defrosting: temperature, 40° F.; time 48 hr.
    Dilution: one part of defrosted egg to one part water, by volume.
    Drying conditions: inlet temp., 250° F.; outlet temperature, 140° F.; except for material dried just after freezing.
    B.F.: before freezing: A.F.: after freezing.

The results of the more comprehensive storage experiment are shown in Fig. 1. Since many of the factors studied had no effect on the quality of the powder, measurements to assess these factors were considered as replicates and average values are shown in the figure.

Powders prepared from frozen incubator rejects and musty eggs were definitely of poor quality as assessed by the fluorescence measurement.

However, the solubility of these powders was better than that of powders from Grade *A*, Grade *C*, or cracked eggs. It is possible that enzymic digestion of the protein occurred in the incubator reject and musty eggs and that these processes made the protein fraction more soluble in potassium chloride solution. Failure to differentiate between powders from Grade *A* and Grade *C* or cracked eggs may be attributable to the limited number of samples used or to the changes occurring during freezing. Previous work has shown that Grade
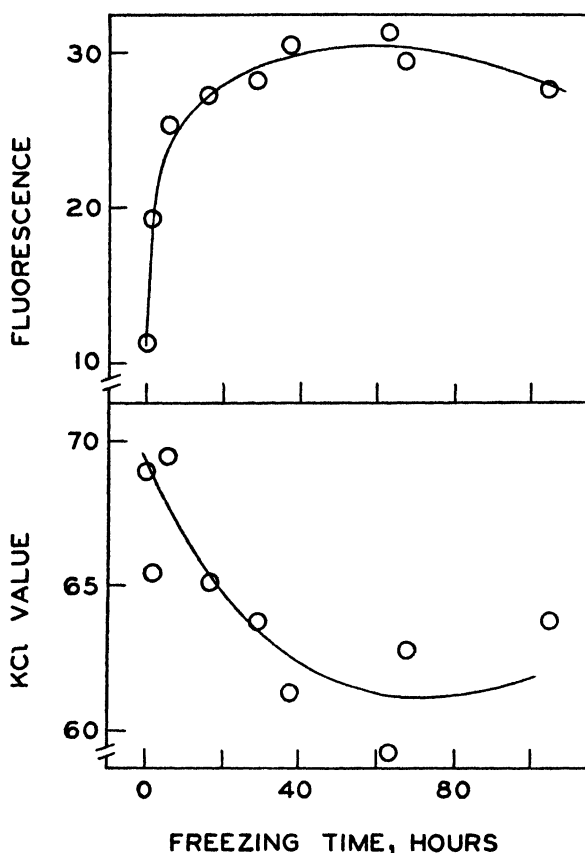
FIG. 2. *The effect of the rate of freezing liquid egg on the quality of the powder produced.*
Defrosting: temperature, 40° F.; time, 48 hr.
Dilution: one part defrosted egg to one part water, by volume.
Drying conditions: inlet temp., 250° F.; outlet temperature, 140° F.

C eggs produce a less desirable powder than Grade *A* eggs (3), and the present experiment indicated that freezing liquid from Grade *A* eggs reduced the quality below that of unfrozen Grade *C* eggs.

The three methods of packaging frozen eggs had no effect on the quality of the powder, and the storage temperatures used affected only the fluorescence values of powder produced from egg stored for 12 months. The values

obtained indicated that storage at $-10°$ F. was preferable, and that there was little difference between temperatures of 0° and $+10°$ F.

Storage time had a greater effect on the fluorescence values than on the potassium chloride values, although in general, the latter measurements showed a marked loss in solubility as a result of the freezing operation: this decrease in solubility has also been observed in commercial practice. The fluorescence values were slightly greater than those encountered in commercial practice and in the preliminary study. It was believed that these high values may have been attributable to freezing or defrosting techniques. These techniques were subjected to further examination.

Egg is usually frozen commercially in 30- or 40-lb. lots and the operation requires from 24 to 72 hr. depending on the method used. In the preliminary experiment, the small blocks of egg used were frozen within a few hours while in the later experiment a much longer time was required. The results of an experiment to evaluate the effect of freezing rates on the quality of the resultant powder are given in Fig. 2 and show that the quality of the powder becomes progressively poorer as the freezing time is increased from 1 to 20 hr., but that further increase in freezing time had little effect on quality.

In commercial practice, frozen egg is usually pulverized or broken into small fragments that may be mixed with water or fresh egg liquid. The temperature of these liquids is not allowed to exceed 45° F. and defrosting is usually completed in one to four hours. In the preliminary experiment, defrosting

TABLE III

EFFECT OF DEFROSTING TEMPERATURE AND TIME ON THE QUALITY OF
POWDER PRODUCED FROM FROZEN LIQUID EGG

(Liquid brought to 40° F.; 15% water added; and dried at inlet
temp. of 250° F. and outlet temp. of 140° F.)

Defrosting		Fluorescence value	Potassium chloride value
Temp., ° F.	Time, hr. (approx.)		
100[1]	1	18 9	78 6
80	10	22 0	72.5
70	18	20 6	74.8
60	28	22.6	70 4
50	40	23 8	73.8
40	48	29 0	76.3

[1] *With stirring and container in contact with water; all others without stirring and containers in contact with air.*

was done in water at 100° F. and completed rapidly, but in the later experiment defrosting was done at 40° F. and required about 48 hr. The results in Table III show that rapid defrosting provided a liquid from which an excellent powder could be produced and that long defrosting times gave powders with high fluorescence values. The solubility of the product was affected by other

factors since high solubilities were obtained from both the most rapidly and the least rapidly defrosted egg.

The foregoing results indicate that frozen egg can be used to produce powder of a satisfactory quality. However, the frozen egg should be stored no longer than six months at temperatures of 10° and 0° (if a longer storage time is necessary, storage temperature should be −10° F.); should be frozen and defrosted in a minimum time, preferably about one hour for each operation; and should have the least possible water added when reducing the viscosity for spraying into the drier.

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The Nutritive Value of Marine Products

XVII. Value of B-Vitamins in Fish Flesh for Growth of Young Rats

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ABSTRACT

The fleshes tested as sources of the B-vitamins for the growth 'of young rats are listed in the order of decreasing value: pork, beef, white spring salmon, halibut, lemon sole, and lingcod. Pork flesh permitted a maximal rate of growth. The principal vitamin deficiencies found in the other fleshes are indicated in decreasing order of magnitude: beef—thiamine, riboflavin; white spring salmon—riboflavin, thiamine; halibut—riboflavin and pantothenic acid, thiamine; lemon sole and lingcod—thiamine, riboflavin, pantothenic acid.

During the last ten years there have been developed with varying degrees of success numerous chemical and microbiological assay methods for a number of the components of the B-vitamin complex. Although such advances have made the need for biological determinations less imperative, nevertheless, as Oser, Melnick and Hochberg (1945) have pointed out elsewhere, the biological method enjoys a unique advantage since it measures the available or potentially effective portion of the vitamin content of the source under examination. The chemical and microbiological methods on the other hand are designed to estimate the total amount of each vitamin present. The value of the latter data is unquestioned. It must be emphasized, however, that there exists no necessary relationship between the levels of vitamins so determined and the physiological availability of these vitamins. As has been found for some of the minerals, accompanying foods have also a marked influence on the availability of certain of the vitamins. There is for example the well known action of raw egg-white which renders biotin unavailable to the body (Sydenstricker, Singal, Briggs, DeVaugh and Isbell 1942). Certain types of live yeast when incorporated into thiamine-rich diets lower the amount of this vitamin available to the organism (Ness, Price and Parsons 1946). The role of thiaminase, present in the raw flesh of certain fish, in the destruction of thiamine and the production of a deficiency of this vitamin may also be recalled (Green, Carlson and Evans 1941). Brown, Thomas and Bina (1946) in a paper on the determination of nicotinic acid concluded that "the drastic treatment required for the liberation of nic-

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otinic acid from natural products is such as to cast doubt on the availability of large proportions of these values in human nutrition". They further believe that a comprehensive biological assay of food materials for available nicotinic acid values would be justified. The experiments herein described constitute an attempt to rate in a general way the value of different fleshes as sources of the B-vitamins for growth. Where certain of the fleshes have been found to be deficient an attempt has been made to determine wherein the deficiency lay. This work was intended to be a preliminary to more detailed study of the B-complex in fish flesh.

At the suggestion of Dr. N. M. Carter, Director of the Pacific Fisheries Experimental Station, four commercially important types of fish were chosen for this study: lingcod (*Ophiodon elongatus*), halibut (*Hippoglossus stenolepis*) lemon sole (*Parophrys vetulus*), and white spring salmon (*Oncorhynchus tshawytscha*). Other flesh preparations included for comparative purposes were those of pork and beef.

EXPERIMENTAL

The flesh samples were processed as described in a previous paper (Beveridge 1946) except that the minced flesh was dehydrated for about two hours at a temperature of not more than 40°C. and finally to a moisture content of approximately 6% at about 32°C. The total drying time varied between 8 and 10 hours. The air used in the drying operation was dried by passing it through a brine coil. The temperature of the circulating medium was −29 to −31°C. At every stage, attempts were made to shield the materials from light. These alterations in technique were made in order to minimize destruction of B-vitamins. Waisman and Elvehjem (1941) have shown that under the conditions used by them for making dried meat samples little or no destruction of vitamins took place. The method used by these workers consisted of spreading the minced meat on large pans and circulating warm air over the latter at a temperature of 35 to 50°C. The milder conditions utilized in the present study should therefore be at least as efficacious in preserving the vitamin content of the substances under investigation. The pork and beef flesh preparations were obtained by processing definite proportions of all the wholesale cuts and were thus roughly representative samples of the respective carcasses. In the two feeding trials described below fresh preparations were made for each trial. As a necessary preliminary task moisture and fat determinations were carried out on each sample. The diets were then made up on the basis of these figures to contain the same amount of moisture-free material and to be of isocaloric value. The rats were of the Wistar strain and in every case they were divided amongst the different groups according to weight, sex, and litter. They were housed in a room at a controlled temperature of 20 to 22°C. in individual cages having ¼ inch-mesh screen floors.

The basal diet was of the following composition: casein (Labco brand, fat-free, vitamin-free) 20%, sucrose 62%, beef dripping 6%, Mazola oil 4%, salts (Beveridge and Lucas 1945) 5%, agar (in first feeding trial only) 2%, celluflour (in second feeding trial only) 2%, cod liver oil (Mead's 1800 I.U. of vitamin A per g. and 175 I.U. of vitamin D per g.) 1%. Vitamin supplements

for control diet A consisted of 5% yeast and 1% Lilly's liver concentrate. These materials were incorporated in place of an equal amount of sucrose. Control diet B contained 0.3% choline chloride and 1% of a mixture of powdered sugar and crystalline vitamins made up so that each gram contained 500γ thiamine hydrochloride, 400γ riboflavin, 200γ pyridoxine hydrochloride, 1000γ calcium pantothenate, and 1000γ nicotinamide.

Feeding Trial 1

The rats, males and females, on reaching 50 ± 2½ g. in weight, were placed on the basal diet for 14 days to deplete them of their stores of B-vitamins. An average weight loss of several grams resulted in most cases from this pre-

Figure 1. Average growth curves of males on diets in which the B-vitamins were supplied by different flesh preparations at a dietary level of 20%.

liminary treatment and where necessary some slight rearrangements were made in the various groups to make them as nearly alike as possible. Five animals were placed on each test diet and the flesh preparations were incorporated into the diets at 20 and 8% levels in place of similar amounts of sucrose. The diets, which were given *ad libitum*, were fed for 28 and 42 days respectively. Five rats were retained on the basal diet to serve as a control to demonstrate the inadequacy of the diet when no vitamins were supplied. Diet A was included to show that when B-vitamins were supplied in adequate amounts, normal growth rates resulted. Diet B was fed to show the rate of growth obtained when only the five crystalline vitamins, mentioned previously, were given.

The average growth curves for the male rats, and female rats on the 20% flesh supplements are presented in figures 1 and 2 respectively. Pertinent data are submitted in table I.

TABLE I. Gains in weight induced in young rats by diets in which the B-vitamins were supplied by 20% of different flesh preparations.

Source of B-vitamins	No. of surviving rats	Sex	Av. init. wt. (g.)	Av. final wt. (g.)	Av. gain in wt. (g.)	Av. daily gain in wt. (g.)	Av. daily food intake (g.)
Pork	1	M	51.1	173.9	122.8	4.4	10.4
	3	F	51.2	167.3	116.1	4.2	10.8
Beef	2	M	46.3	119.2	72.9	2.6	6.7
	3	F	49.4	105.7	56.3 .	2.0	6.5
White spring salmon	2	M	48.5	71.8	23.3	0.8	4.4
	3	F	51.3	85.4	34.1	1.2	5.1
Halibut	2	M	49.4	46.4	−3.0	−0.1	3.5
	3	F	49.8	54.5	4.7	0.2	3.6
Lemon sole	*1	M	52.5	44.2	−8.3	−0.3	2.6
	*2	F	50.3	39.8	−10.5	−0.4	2.2
Lingcod	*1	M	53.1	43.6	−9.5	−0.3	1.7
	3	F	48.4	40.6	−7.8	−0.3	2.5
(A) 5% yeast + 1% liver conc.	3	M	50.2	212.7	162.5	5.8	12.8
	2	F	43.5	136.5	93.0	3 3	9.3
(B) 1% vitamin mixture	3	M	48.3	159.0	110.7	4.0	10.9
	2	F	46.5	129.5	83 0	3.0	9.3

*One rat died.

Pork flesh proved to be by a wide margin the best source of the B-vitamins. Beef flesh proved to be a mediocre source but better than the white spring salmon preparation. Halibut, lingcod, and lemon sole were of negligible value in supplying the B-vitamins.

The rats on diets in which the B-complex was supplied by flesh at 8% levels, with the exception of those on the pork flesh ration and one individual on the beef flesh diet, all lost weight and died before the end of the experiment. The animals on the diet containing 8% pork flesh grew almost as fast as did those on the diet containing 20% beef flesh. The respective gains for the first 28 days on the test diets were for the males 2.6 and 1.8 g. per day, and for the females 1.5 and 2.0 g. per day. The animals retained on the basal diet all died within 48 hours of each other on about the eighteenth day of the test period. This result indicated that no significant amounts of the B-vitamins were contained in the basal diet. The males on the adequate control diet A gained almost 6 g. per rat per day, and the females gained between 3 and 4 g. per day. Slightly lower gains were recorded for the animals on diet B in which the only vitamins supplied were those contained in a vitamin-sugar mixture the dietary level and

composition of which has been previously mentioned. It therefore appears that under these conditions vitamins other than those supplied are required to evoke a maximal growth response.

FEEDING TRIAL 2

This experiment, which was carried out about a year after feeding trial 1, was designed first of all to see whether or not the results of the previous work
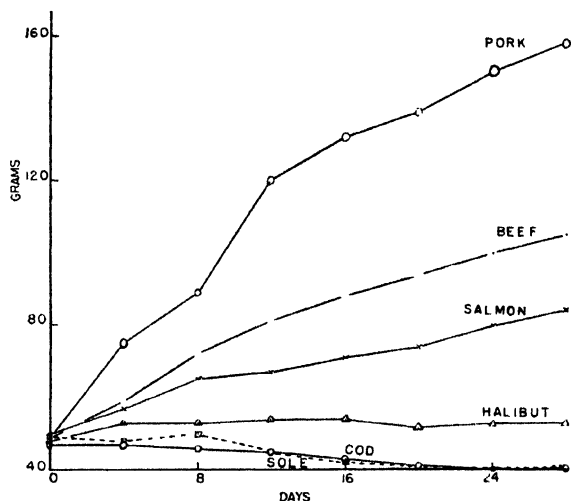
FIGURE 2. Average growth curves of females on diets in which the B-vitamins were supplied by different flesh preparations at a dietary level of 20%.
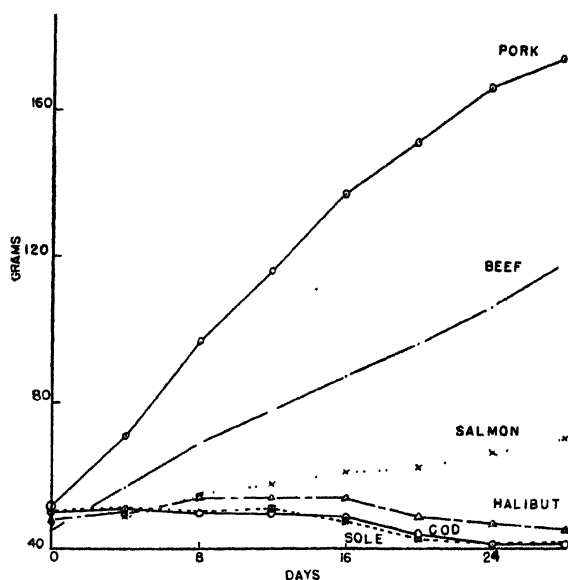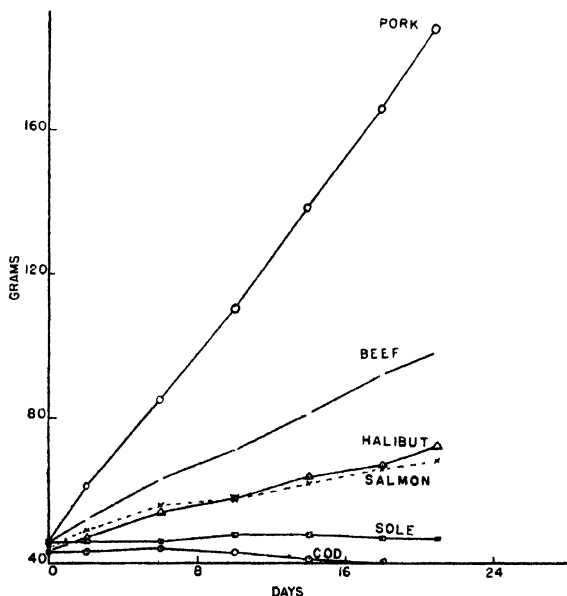

FIGURE 3. Average growth curves of males on diets in which the B-vitamins were supplied by different flesh preparations at a dietary level of 20%.
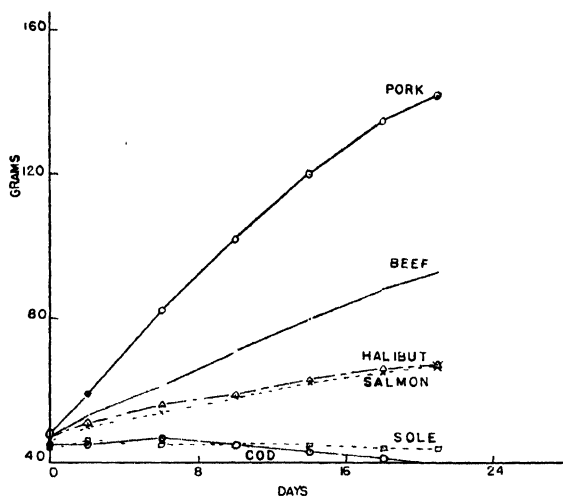
FIGURE 4. Average growth curves of females on diets in which the B-vitamins were supplied by different flesh preparations at a dietary level of 20%.

could be duplicated, and secondly to determine in which vitamins the flesh preparations were deficient. The conditions of the experiment were exactly as described for feeding trial 1 except that the test period was 21 days, five males and five females were placed on each diet, and the flesh preparations were tested only at the 20% level.

The results obtained confirmed those derived from feeding trial 1. The similarity of the growth curves is evident from a comparison of figures 1 to 4

TABLE II. Gains in weight induced in young rats by diets in which the B-vitamins were supplied by 20% different flesh preparations.

Source of B-vitamins	No. of rats	Sex	Av. init. wt. (g.)	Av. final wt. (g.)	Av. gain in wt. (g.)	Av. daily gain in wt. (g.)	Av. daily food intake (g.)
Pork	5	M	47.6	185.1	137.5	6.5	13.0
	5	F	49.8	142.3	92.5	4.4	11.5
Beef	5	M	45.8	99.6	53.8	2.6	6.6
	5	F	46.9	93.7	46.8	2.2	7.0
Halibut	5	M	44.1	73.2	28.8	1.4	4.8
	5	F	46.4	68.4	22.0	1.0	4.8
White spring salmon	5	M	46.6	69.6	23.3	1.1	4.7
	5	F	48.1	69.5	21.4	1.0	4.9
Lemon sole	5	M	47.9	47.5	−0.4	0.0	3.4
	5	F	45.5	41.8	−0.7	0.0	3.7
Lingcod	5	M	44.7	37.6	−7.1	−0.3	2.8
	*4	F	47.4	39.0	−8.4	−0.4	3.1

*One rat died.

and demonstrates that the results obtained are reproducible and therefore valid in a relative sense at least. The growth induced by the halibut diet is definitely greater than that observed in the first experiment. The relevant data of the feeding experiment are given in table II.

It is interesting to note that the diet containing pork flesh induced a maximal growth rate.

In order to determine approximately wherein the different flesh preparations were deficient, different combinations of the five crystalline vitamins incorporated in control diet B were injected daily for 10 days into the rats at the completion of the regular test period of 3 weeks. The same vitamin injections were given to similarly numbered males and females of each group. The series, referred to as 1—5, was comprised of five pairs, one male and one female in each pair. All rats received 50 γ thiamine hydrochloride. In addition, number one rat of each group was given 40 γ riboflavin, number two rat 25 γ pyridoxine hydrochloride, number three rat 100 γ calcium pantothenate, number four rat 100 γ nicotinic acid, and number five rat 40 γ riboflavin and 25 γ pyridoxine hydrochloride. The vitamins were dissolved or suspended in 0.9% saline so that 0.1 ml. contained the amount of each vitamin injected daily.

In addition to the animals already described being given the test diets, two male rats, termed X and Y, were placed on each diet at the end of the preliminary 14-day depletion period and given daily injections of different vitamins. The appellations given to these two series are X_1 and Y_1 for the first 21-day test period, and X_2 and Y_2 for the last 10-day test period. The X_1 rats received 50 γ thiamine hydrochloride daily, and the Y_1 rats received 40 γ riboflavin, 25 γ pyridoxine hydrochloride, 100 γ calcium pantothenate and 100 γ nicotinamide. In the 10 days following the regular test period rats termed X were given, in addition to the B_1, an additional supplement consisting of B_2, pyridoxine hydrochloride and calcium pantothenate; the rats termed Y were given, in addition to the vitamins previously given, thiamine hydrochloride. The data concerning weight gains and food consumption of the rats during the period in which the vitamin supplements were administered are shown in tables III—VIII.

DISCUSSION

An examination of the daily gains in weight (see tables III—VIII) brought about in the rats by different combinations of injections gives some idea wherein the worst vitamin deficiencies lie. The fleshes will be considered in the order of decreasing value as a source of the B-complex for growth.

PORK FLESH

In no case did the addition of any combination of vitamins bring about an increased rate of growth (table III) and since the unsupplemented flesh induced growth comparable to that observed in the animals on the positive control diet (A) (see tables I and II), it may be assumed that a diet containing 20% pork flesh supplies adequate amounts of B-vitamins to promote a maximal rate of growth in rats in the period under examination.

BEEF FLESH

When thiamine was added to rat X_1 (table IV) the rate of growth was about the maximum. On the other hand the addition of all the other vitamins to rat Y_1 brought about no increase in growth over that recorded for the animals on the unsupplemented diet (table I, II). This result indicates that thiamine is the chief vitamin limiting growth in the diet containing beef flesh. In the series 1—5 (table IV) in which thiamine was given along with different com-

TABLE III. Effect of various vitamin supplements on the ability of a diet containing 20% pork flesh as a source of B-vitamins to promote growth in young rats.

Rat no.	Vitamin supplement	Sex	Init. wt. (g.)	Final wt. (g.)	Gain in wt. (g.)	Daily gain in wt. (g.)	Daily food intake (g.)
1	$B_1 + B_2$	M	180.2	223.9	43.7	4.4	15.9
		F	144.5	161.1	16.6	1.7	13.0
2	$B_1 +$ pyridoxine HCl	M	187.7	244.2	56.5	5.7	18.8
		F	140.6	156.6	16.0	1.6	13.1
3	$B_1 +$ Ca pantothenate	M	179.0	226.0	47.0	4.7	18.6
		F	143.9	163.0	19.1	1.9	12.3
4	$B_1 +$ nicotinamide	M	182.5	231.2	48.7	4.9	14.9
		F	124.9	146.5	21.6	2.2	11.5
5	$B_1 + B_2 +$ pyridoxine HCl	M	196.0	237.2	41.2	4.1	16.9
		F	157.5	172.2	14.7	1.5	12.2
X_2	As above + Ca pantothenate	M	174.3	225.1	50.8	5.1	17.3
Y_2	As above + nicotinamide	M	181.7	219.4	37.7	3.8	16.2
X_1	B_1	M	51.3	174.3	123.0	5.9	11.7
Y_1	$B_2 +$ pyridoxine HCl + Ca pantothenate + nicotinamide	M	47.6	181.7	134.1	6.4	14.3

binations of the other vitamins, essentially the same gain was recorded for all with the exception perhaps of those rats to which riboflavin was also given. It therefore appears that the beef flesh preparation used was definitely deficient in thiamine and perhaps slightly deficient in riboflavin.

SALMON FLESH

An examination of table V reveals that neither the B_1 given to rat X_1 nor the other four crystalline vitamins given to rat Y_1 evoked a maximal growth response, although in the latter case the growth rate approached normal. This indicated that the salmon flesh diet constituted a fair source of B_1 and that

some one or all of the other vitamins were slightly deficient. However, the results of the series, 1—5, X_2, Y_2, indicated that only when B_1 and B_2 were given together was a maximal rate of growth obtained and the rate of growth was as great when only B_1 and B_2 were given as when all the vitamins were administered. These data reveal that the principal deficiency in salmon flesh is riboflavin and that, although it is a relatively good source of thiamine, the amount in a diet containing 20% salmon flesh is not quite sufficient to promote a maximal rate of growth.

TABLE IV. Effect of various vitamin supplements on the ability of a diet containing 20% beef flesh as a source of B-vitamins to promote growth in young rats.

Rat no.	Vitamin supplement	Sex	I nit.wt. (g.)	Final wt. (g.)	Gain in wt. (g.)	Daily gain in wt. (g.)	Daily food intake (g.)
1	$B_1 + B_2$	M	73.9	140.8	66.9	6.7	11.2
		F	104.6	143.5	38.9	3.9	12.0
2	$B_1 +$ pyridoxine HCl	M	110.0	166.3	56.3	5.6	12.9
		F	129.4	158.5	29.1	2.9	12.6
3	$B_1 +$ Ca pantothenate	M	86.4	138.9	52.5	5.3	10.6
		F	110.5	154.7	44.2	4.4	13.7
4	$B_1 +$ nicotinamide	M	123.0	181.0	58.0	5.8	13.4
		F	48.2	85.3	37.1	3.7	8.3
5	$B_1 + B_2 +$ pyridoxine HCl	M	104.9	171.3	69.4	6.9	14.1
		F	75.6	88.4	12.8	1.3	11.0
X_2	As above + Ca pantothenate	M	151.2	206.8	55.6	5.6	12.3
Y_2	As above + nicotinamide	M	92.8	148.2	55.4	5.5	13.0
X_1	B_1	M	41.1	151.2	110.1	5.2	10.0
Y_1	$B_2 +$ pyridoxine HCl + Ca pantothenate + nicotinamide	M	53.7	92.8	39.1	1.9	6.5

HALIBUT

The addition of B_1 alone (X_1, table VI) brought about only a slight increase in the rate of growth over that observed on the animals given the unsupplemented flesh diet. The injection of the four other vitamins (Y_1) elicited weight gains which were just slightly subnormal, thus indicating that the halibut preparation was a fair source of vitamin B_1. In the series 1—5 injections of B_1 plus any one of the other vitamins brought about increased gains in weight which were definitely subnormal compared to the maximal growth rate. When B_1 plus B_2 were given, the gains were greater than was obtained with any other combination of two vitamins. Since the addition of pyridoxine to a combination of B_1 plus

B_2 brought about no increase in weight gains, it may be assumed that halibut flesh is an adequate source of this vitamin.

When calcium pantothenate was added to this combination a definite increase in the rate of growth was observed, indicating that this vitamin had been a limiting factor in growth (see rat X_2). When nicotinamide was added to the combination (see rat Y_2) a further slight increase of doubtful

TABLE V.   Effect of various vitamin supplements on the ability of a diet containing 20% salmon flesh as a source of B-vitamins to promote growth in young rats

Rat no.	Vitamin supplement	Sex	Init. wt. (g.)	Final wt. (g.)	Gain in wt. (g.)	Daily gain in wt. (g.)	Daily food intake (g.)
1	$B_1 + B_2$	M	79.7	146.4	66.7	6.7	11.4
		F	74.9	134.7	59.8	6.0	12.1
2	B_1 + pyridoxine HCl	M	80.6	122.7	42.1	4.2	8.7
		F	72.2	102.1	29.9	3.0	8.0
3	B_1 + Ca pantothenate	M	73.1	109.5	36.4	3.6	8.0
		F	75.4	109.3	33.9	3.4	8.9
4	B_1 + nicotinamide	M	62.4	97.4	35 0	3.5	7.5
		F	62.3	84.1	21.8	2.2	6 4
5	$B_1 + B_2$ + pyridoxine HCl	M	53.8	104.1	50.3	5.0	8.2
		F	62.5	97.6	35.1	3.5	9.1
X_2	As above + Ca pantothenate	M	92.3	153.6	61.3	6.1	12.3
Y_2	As above + nicotinamide	M	128.3	182.5	54.2	5.4	15.4
X_1	B_1	M	44.5	92.3	47.8	2.3	6.2
Y_1	B_2 + pyridoxine HCl + Ca pantothenate + nicotinamide	M	37.1	128.3	91.2	4.3	8 7

significance was observed.   One may therefore conclude that the halibut flesh seemed to be adequate in pyridoxine and perhaps in nicotinamide, whereas thiamine, riboflavin, and pantothenic acid were present in sub-optimal amounts for maximal growth.

LEMON SOLE

When only thiamine was added the rate of growth was extremely slow (rat X_1, table VII) but without it the rats actually lost weight (table II).   The addition of all the other vitamins without thiamine resulted in a loss of weight, thus indicating the low level of the latter vitamin in this preparation.   The addition of B_1 and B_2 evoked a rate of growth which was only slightly subnormal.

The two chief limiting factors in growth therefore appear to be the deficiency of these two vitamins in the lemon sole flesh. The injection of pyridoxine along with these two vitamins caused no apparent difference in growth rate and indicates that pyridoxine is probably present in adequate amounts. When calcium pantothenate was added to the latter combination of vitamins maximal growth ensued. It appears therefore that lemon sole flesh is grossly deficient in B_1, and deficient in riboflavin and pantothenic acid. The evidence indicated that pyridoxine and nicotinamide are present in sufficient quantity.

TABLE VI. Effect of various vitamin supplements on the ability of a diet containing 20% halibut flesh as a source of B-vitamins to promote growth in young rats.

Rat no.	Vitamin supplement	Sex	Init. wt. (g.)	Final wt. (g.)	Gain in wt. (g.)	Daily gain in wt. (g.)	Daily food intake (g.)
1	$B_1 + B_2$	M	81.1	122.0	40.9	4.1	9.2
		F	59.1	96.3	37.2	3.7	8.2
2	B_1 + pyridoxine HCl	M	75.2	108.0	32.8	3.3	7.6
		F	86.2	109.0	22.8	2.3	8.4
3	B_1 + Ca pantothenate	M	71.8	104.5	32.7	3.3	7.4
		F	66.7	101.5	34.8	3.5	9.6
4	B_1 + nicotinamide	M	74.0	102.3	28.3	2.8	6.6
		F	67.2	94.3	27.1	2.7	9.4
5	$B_1 + B_2$ + pyridoxine HCl	M	64.1	105.0	40.9	4.1	8.0
		F	62.8	101.3	38.5	3.9	8.9
X_2	As above + Ca pantothenate	M	97.9	154.7	56.8	5.7	12.9
Y_2	As above + nicotinamide	M	127.4	192.6	65.2	6.5	15.9
X_1	B_1	M	51.4	97.9	46.5	2.2	7.3
Y_1	B_2 + pyridoxine HCl + Ca pantothenate + nicotinamide	M	45.2	127.4	82.2	3.9	9.1

LINGCOD

A study of tables VII and VIII shows the existence of a marked similarity between the data obtained for lingcod and lemon sole, and the conclusions reached for the latter may be applied also to the former.

The question of the effect of differences in the amino acid composition of the proteins of the fish, beef and pork preparations on the requirement of B-vitamins has been raised. Although it is true that the amino acid composition of these substances differs slightly, nevertheless, the possibility is extremely faint that these slight differences, superimposed on a diet already adequate in

protein, would exert any demonstrable effect on the requirement of the B-vitamins.

In every case when the five crystalline vitamins used in this study were given as a supplement to flesh diets maximal growth was obtained. This result indicates that the flesh preparations supplied adequate amounts of the other factors required to promote a maximal growth rate. That such metabolites are required may be revealed by comparing the respective gains induced by control diets A and B (cf. table I).

TABLE VII.   Effect of various vitamin supplements on the ability of a diet containing 20% lemon sole flesh as a source of B-vitamins to promote growth in young rats.

Rat no.	Vitamin supplement	Sex	Init. wt. (g.)	Final wt. (g.)	Gain in wt. (g.)	Daily gain in wt. (g.)	Daily food intake (g.)
1	$B_1 + B_2$	M	48.9	88.7	39.8	4.0	7.2
		F	45.9	77.0	31.1	3.1	6.6
2	B_1 + pyridoxine HCl	M	43.7	65.1	21.4	2.1	5.8
		F	44.1	61.7	17.6	1.8	5.4
3	B_1 + Ca pantothenate	M	44.1	61.7	17.6	1.8	4.4
		F	47.7	64.4	16.7	1.7	4.9
4	B_1 + nicotinamide	M	56.4	71.6	18.2	1.8	5.0
		F	39.1	60.6	21.5	2.2	5.7
5	$B_1 + B_2$ + pyridoxine HCl	M	44.3	86.0	41.7	4.2	6.9
		F	47.2	85.6	38.4	3.8	7.7
X_2	As above + Ca pantothenate	M	81.9	140.8	58.9	5.9	11.9
Y_2	As above + nicotinamide	M	50.2	102.4	52.2	5.2	9.1
X_1	B_1	M	47.4	81.9	34.5	1.6	5.5
Y_1	B_2 + pyridoxine HCl + Ca pantothenate + nicotinamide	M	45.9	50.2	4 3	0.2	4.4

SUMMARY

The relative values of the fleshes of lingcod, lemon sole, halibut, white spring salmon, beef, and pork at the 20% level as sources of the B-vitamins have been determined twice using the growth rate of young rats as a criterion.

Pork flesh permitted a maximal rate of growth. The other fleshes evoked subnormal growth responses and are placed in decreasing order of adequacy for growth with respect to the B-vitamins: beef, white spring salmon and halibut. The feeding of lemon sole and lingcod brought about weight losses.

The principal deficiencies in the different fleshes as determined by the effects of the injection of various combinations of vitamins are indicated in

decreasing order of magnitude: beef—thiamine, riboflavin; salmon—riboflavin, thiamine; halibut—riboflavin and pantothenic acid, thiamine; lemon sole and lingcod—thiamine, riboflavin and pantothenic acid.

The group of fish flesh tested compared most unfavourably with pork and beef flesh as sources of the B-vitamins for the growth of young rats.

TABLE VIII. Effect of various vitamin supplements on the ability of a diet containing 20% lingcod flesh as a source of the B-vitamins to promote growth in young rats.

Rat no.	Vitamin supplement	Sex	Init. wt. (g.)	Final wt. (g.)	Gain in wt. (g.)	Daily gain in wt. (g.)	Daily food intake (g.)
1	$B_1 + B_2$	M	37.3	76.5	39.2	3.9	6.9
		F	42.7	71.3	28.6	2.9	6.5
2	B_1 + pyridoxine HCl	M	35.6	57.0	21.4	2.1	4.5
		F	39.5	52.3	12.8	1.3	4.3
3	B_1 + Ca pantothenate	M	39.7	57.3	17.6	1.8	5.1
		*F					
4	B_1 + nicotinamide	M	40.8	58.9	18.1	1.8	4.2
		F	38.3	51.5	13.2	1.3	4.3
5	$B_1 + B_2$ + pyridoxine HCl	M	34.4	63.3	28.9	2.9	4.9
		F	35.3	57.0	21.7	2.2	4.6
X_2	As above + Ca pantothenate	M	76.2	140.4	64.2	6.4	11.0
Y_2	As above + nicotinamide	**M					
X_1	B_1	M	39.3	76.2	36.9	1.8	4.8
Y_1	B_2 + pyridoxine HCl + Ca pantothenate + nicotinamide	M	45.5	34.9	−10.6	−0.5	3.4

*Died during the preliminary 21-day test period.
**Died during the 10-day test period.

ACKNOWLEDGMENTS

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Fully Automatic Test Laboratory Holds –70 F

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THE war brought demands for low temperature testing of many types of service equipment and supplies. In Canada many such tests were referred to the National Research Laboratories. At the outset the low temperature laboratories in the Division of Applied Biology, originally designed for storage studies on perishable foods, provided temperatures down to –40 F. As the war progressed, temperatures as low as –70 F were required and new facilities had
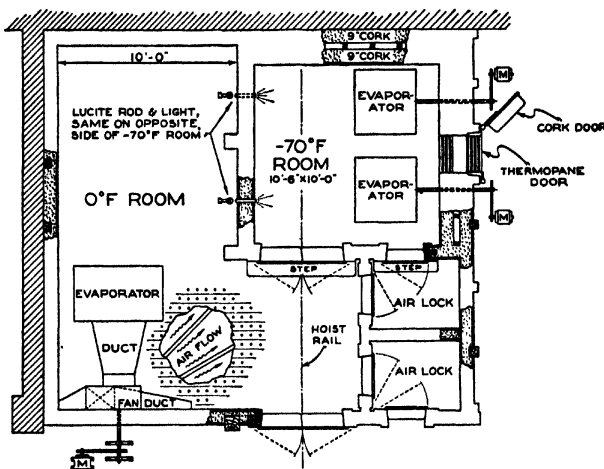
Fig. 1. Plan view of 0 F and –70 F laboratory rooms, showing arrangement of equipment and facilities.

to be provided. The requirements were a cold laboratory about 10 by 10 by 10 feet, providing temperatures from –40 to –70 F, and capable of continuous operation under fully automatic control, so that tests could be conducted with a minimum of delay. The original design, together with modifications necessitated either by shortages of equipment or to improve operating performance, is described in this article.

Design Conditions

The Freon refrigerants were preferred to ammonia since they require lighter equipment, smaller evaporators, and more easily obtained controls. In fact, various members of the Freon family have formed the basis of modern installations of comparable size and similar performance. [1,2,5]

The compression ratio necessary to obtain the de-

sired temperatures demanded a multistage or cascade system. These systems have been compared by Sloan[7] and Carter[3] with the type of controls described by Newton[6]. The use of two independent single-stage systems arranged in cascade was considered more amenable to accurate and reliable automatic control, this being considered of greater importance for experimental purposes than the higher efficiency of the multistage systems.

Although the cascade system has the disadvantage of imposing a temperature differential between the high side of one system and the low side of the other, resulting in an overall increase in compression ratio and horsepower requirements, this can be partially offset by the use of two different refrigerants. The use of Freon-12 in the high and Freon-22 (monochlorodifluoromethane) in the low temperature stage would take advantage of the higher vapor pressure and greater refrigerating effect of F-22 yet maintain the advantages of F-12 in the high temperature stage.

A direct expansion system was indicated on the grounds of simplicity and performance. This demanded a method of temperature control that was both accurate and reliable in operation. Previous experience indicated that the conventional off-on controls were not sufficiently accurate to cope with the wide variation in heat loads met in experimental tests. Auxiliary controls might have improved performance but it was felt that these should be avoided in the interest of simplicity, particularly since their performance under low temperature conditions was doubtful. The duties demanded partly flooded evaporators operating at low pressures, and thereby increased the risk of liquid splash-over to the compressor head when starting under off-on control.

The difficulties were avoided by adopting a modulating system of control in which the compressor operates continuously. The simplest system applicable to the available compressors was to vary their speed continuously in accordance with the load. Previous experience had shown that this type of control was satisfactory in principle but the use of direct current motors, or variable speed drives of the belt-and-pulley type, under automatic control, was not entirely reliable and required considerable servicing. Consequently a fluid drive capable of continuously variable output speed and maximum output torque at all operating speeds was chosen.

To provide the necessary accuracy, range, and ease and precision of setting required, resistance-thermometer equipment was chosen for the temperature control. In conjunction with a recording controller of suitable range, this provided for both temperature recording and controlling. The control mechanism acted by adjusting the variable speed drive in accordance with the temperatures. Controllers of this type permitted other essential or desirable features to be incorporated to obtain maximum operating flexibility. Simple switching permitted: selection of either manual or automatic control; establishment of a definite compression ratio between the compressors by the control of the second compressor at a fixed percentage of the speed of the first; manual setting to control the temperature range over which full modulation occurred; and determination of the rate at which the controller

Table 1. Refrigerating Load[1]

0 F Room				—70 F Room		
Design Load				Design Load		
3 kw experimental load	10,250			2 kw experimental load	6830	
2 men in room	1200			2 men in room	1200	
1—½ hp fan (motor outside)	1910			1—¼ hp motor for circul. pump	640	
4—60-watt lights	840	14,200		2—¾ hp fans (motors outside)	1910	10,580
Heat Leakage				Heat Leakage		
Gain from outside	6800			Walls	2200	
Loss from inside	—2200			Doors, sleeves, fan shafts, etc.	720	2920
Door, sleeves, fan shafts, etc.	1200	5,800				
Total—Btu per hour		20,000		Total—Btu per hour		13,500

adjusted the compressor speed with load fluctuations (droop control).

Description of Rooms

Operation of a —70 F laboratory in a heated building meant that the temperature differential across the walls might reach 150 F. To provide additional refrigerated space at a higher temperature, split the large temperature differential, and minimize the defrosting problem, the —70 F laboratory was constructed in a larger insulated room designed to operate at about 0 F. The layout of the room, entrance doors, air locks, and other facilities are shown in Figure 1.

The outer room was approximately 24 by 22 by 12 ft, insulated throughout with nine inches of cork. The fan, driven by a multispeed motor outside the room, had a capacity of 2300 cfm at 950 rpm and 1 in. suction pressure. Air from the room was drawn through an evaporator suspended from the ceiling, and discharged beneath a false floor. Part of the air passed through a 3-in. space separating the floor, outside walls, and ceiling of the low temperature room from the walls of the outer room, and the remainder passed up through perforations in the false floor. The estimated refrigerating load for this room appears in Table 1.

The low temperature room was about 10½ by 10 by 9 ft and was separated from the outer room by nine inches of insulation. To reduce the heat gain the fan motors and lights were mounted outside. Four lucite rods 2 in. in diameter were placed in the walls at the 5½ ft level as indicated in the drawing. The outer surfaces of these rods were polished and lighted individually with reflector type light bulbs. The inner surface of each rod was tapered and projected into the room. These provided sufficient light for all ordinary purposes. The estimated refrigerating load for this room also appears in Table 1.

Certain additional facilities and services were required for the experimental work. A trolley truck and wide doors served to admit large or heavy pieces of equipment. A window was provided to permit observations to be made from the warm laboratory. This window consisted of six layers of double glass (Thermopane) or 12 sheets in all, each sheet separated by an air space. The inner six sheets were fixed, but the outer six were carried in a hinged frame. When not in use this hinged portion could be swung out and replaced by a cork insulated door. In practice this assembly has never frosted over to an extent that prevented satisfactory observations. Other services included ac and dc power and a two-way communication system.

Apart from the method of temperature control and the evaporator, the 0 F room was cooled by a conventional single stage F-12 condensing unit. To permit the same accuracy and flexibility of control in this as in the —70 F room, a resistance thermometer and compressor speed control were employed. This equipment was similar to that described later in connection with the —70 F room.

The evaporators were designed for electrical defrosting, incorporating a method developed in connection with some emergency refrigeration installations on cargo vessels.[4] In this earlier application other defrosting methods could not be used. Electrical heaters placed beneath the coil or attached to a localized part of the surface were found to be comparatively inefficient. By replacing two adjacent, vertical fins on equally spaced centers across the coil with two 1/16-in. copper plates (3/16 in. apart), a space was provided for inserting flattened tubular heating elements. Thus, with the heaters in direct contact with the plates and the tubes expanded into the plates and fins when fabricated, the heat was uniformly distributed across the width and depth of the coil. This design had proved satisfactory and was adopted.

The extended surface evaporator used in the 0 F room had a face area of 7.5 sq ft (30 x 36 in.). The tubes were 10 deep, 15 high and arranged in 10 vertically parallel circuits, expanding into 48 aluminum fins per foot plus the paired copper plates on 7.25-in. centers. Three 400-watt heaters were fitted between each pair of plates. The frame of the evaporator was insulated with 2 in. of cork and the face next to the room was fitted with a hinged door of equivalent insulating value. Defrosting was performed manually by
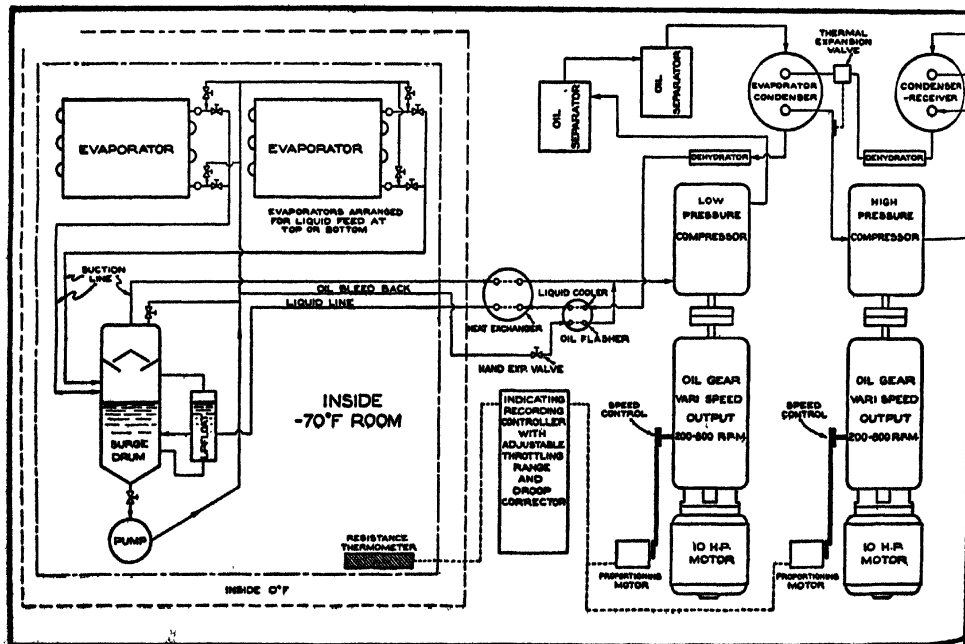
Fig. 2. Schematic diagram of cascade refrigerating system for —70 F room.

Fig. 3. View in machinery room showing the three drive units and compressors for the 0 F and —70 F rooms.

stopping the compressor and fan, closing the door, and applying 6000 watts to the heaters. The coil was usually free from frost in 45 min during which time the room temperature seldom rose more than 5 F.

Refrigerating System —70 F Room

The layout of the cascade refrigeration system for the —70 F room is shown in Figure 2.

High Pressure Condensing Unit--This compressor was a standard reciprocating F-12 unit with forced oil circulation and having a displacement of 2200 cu ft per hour at 660 rpm. The water-cooled condenser-receiver was of the shell-and-tube type, with short fins on the tubes to facilitate cooling the liquid refrigerant. The expansion valve was of the conventional thermal expansion type. The evaporator-condenser between the two stages was identical with the water cooled condenser-receiver. It was arranged for partial flooding of the tubes with the high pressure refrigerant (F-12) and condensation of the low pressure refrigerant (F-22) in the shell.

Low Pressure Condensing Unit—This compressor was also a standard reciprocating F-12 unit with forced oil circulation, having a displacement of 3900 cu ft per hour at 660 rpm. The refrigerant from this machine passed through two oil separators before entering the condenser-evaporator described above. The liquid refrigerant first passed through a heat exchanger, then through a float valve controlled by the liquid level in an expansion tank. The flash gas generated in cooling the liquid passed directly back to the compressor.

The original design contemplated the installation of a small constant displacement circulating pump to force the liquid refrigerant from the expansion tank near floor level to the ceiling-mounted evaporators. The headers on the evaporators were arranged so that the liquid could be fed to the lower header with suction from the upper header or vice versa. The suction line returned to the expansion tank fitted with baffle plates for the removal of entrained liquid.

Any oil that passed the separators would naturally collect in the expansion tank. To maintain a constant oil return to the compressor an auxiliary liquid line from the expansion tank was brought to an auxiliary

heat exchanger and then to the compressor. This insured that the oil and a small amount of refrigerant would be returned directly to the compressor.

Two identical evaporators were provided for this operation to permit defrosting of one while the other remained in operation. These evaporators each had a face area of 3.33 sq ft, 10 tubes deep and 10 high. All tubes were served by common headers at the top and bottom—i.e., 10 vertically parallel circuits. The coils carried 36 aluminum fins per foot, in addition to the paired copper plates on 6 in. centers as described previously for the reception of electrical heaters. The
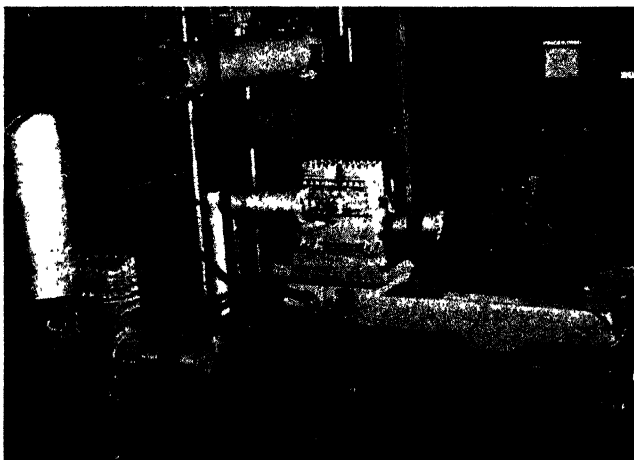
Fig. 4. Closeup of refrigerating equipment shows electric motor, varispeed transmission and compressor directly coupled.

heaters employed permitted 4800 watts to be used on each evaporator. Insulated hinged doors were provided for both faces of the evaporator and the frames carried 2 in. of cork insulation.

The air wheel fans used on these evaporators were provided with shafting that extended through sleeves in the walls. Belt drives from multispeed motors permitted a considerable variation in air movement. Maximum circulation rates were 4500 cfm per fan, but while both evaporators are in operation the conditions can readily be maintained with a total air flow of 2000 cfm.

Controls

The temperature control equipment consisted of resistance thermometers with recorder-controllers (Leeds and Northrup) operating a variable speed fluid drive (Oil Gear Company). The controllers provided for either manual or fully automatic operation with a manual throttling range control and a time delay setter.

Manual operation permitted each compressor to operate at a constant speed selected by the operator. This type of operation was always used when cooling the room from an initial temperature of 0 F or higher. The high pressure unit was first brought to full speed and allowed to operate for a short period; the low temperature unit was then started with the speed being gradually increased by manual control. When full speed was attained the controllers were set for auto-

matic operation. Ordinarily the temperature of the test room was considerably below ambient conditions and the period of manual operation was either unnecessary or of short duration.

Under automatic control each compressor was on a separate branch of the main control circuit. One, the primary, was activated directly by the controller. The secondary circuit, activated by the primary, maintained the second compressor at a predetermined percentage of the speed of the compressor operated by the primary. Manual switching selected the compressor activated by the primary circuit and the speed ratio of the second machine. In practice the low temperature compressor was activated by the primary control circuit thus providing for an immediate change in compressor capacity, by means of speed regulation, should the control circuit call for adjustment. If the primary control were set to operate the high temperature unit —i.e., to lower the evaporator-condenser temperature and thereby increase the refrigerating effect of the low temperature unit—a time lag in temperature adjustment, with attendant undesirable fluctuations in the controls, would occur due to the residual heat retaining capacity of the evaporator-condenser. The overall compression ratio between the two cascade units was a function of condensing water temperature, the temperature of the low pressure evaporator, and the refrigerant used, therefore manual setting of the percentage speed to be maintained by the secondary circuit was dependent upon these factors. With F-12 in both cascade units and the room operating at —70 F, equal compression loads could be maintained at a speed ratio of high temperature unit to low temperature unit of 1:2.

When the desired temperature had been set on the controller, any departure from this value unbalanced the bridge circuit containing the resistance thermometer. This unbalance was converted into mechanical motion setting the recorder pen and adjusting the compressor speed through a proportioning motor positioning the control lever on the Oil Gear. Subsequent speed changes followed this preset sequence for both compressors. Manual adjusting of the modulating range and time delay setting permitted precise temperature adjustment during test periods when the loads varied greatly and could be set to avoid "hunting" when loads were heavy or precise control was unnecessary.

Equipment As Installed

As the cold laboratory had to be placed in operation without undue delay, wartime shortages of materials necessitated some modifications. It was impossible to obtain delivery of a satisfactory pump for circulating the liquid refrigerant. This pump had to function over temperature ranges extending from ordinary room temperature down to —90 F or lower and pressure variations from 200 psi to 29 in. vacuum. Available types had inadequate sealing glands or seized at the low temperatures. The room was put in operation by by-passing the float valve and surge drum and using a hand valve as an expansion valve. Later this was supplemented by a Detroit Lubricator differential temperature expansion valve. The low temperature stage was charged with F-12 during these changes and, as it has

provided the temperature conditions required, the low side has not yet been charged with F-22.

The first year of operation revealed a number of minor mechanical difficulties. The head on the conventional water cooled condenser modified as a condenser-evaporator for use between the units was originally fitted with lead gaskets. These leaked after several operating cycles and had to be replaced with Neoprene gaskets. The rubber in the diaphragm-type hand valves exposed to the low temperature cracked when adjustments were made at operating temperatures. This was remedied by using Neoprene gaskets on the valves to stop leakage.

At the low back pressures encountered at the design temperature, the suction valve reseating springs used in normal operation were removed at the outset. Under these conditions the valves stuck open owing to contact established by the oil film. Punch marks on the ring valves failed to solve the problem. This difficulty was finally overcome by fitting permanent points on the under surface of the valve guide seat to break the oil film.

Other difficulties of a service nature have occurred occasionally. The mechanical seals on the drive shaft of the low temperature compressor have leaked air after long periods of low temperature operation. Also the screens in the dehydrators have become plugged and have subsequently collapsed. These difficulties have not caused much loss of operating time and may be partly remedied when the low temperature system is charged with F-22 and other contemplated modifications are made.

Performance

Detailed figures on performance will be reported in a later paper when the tests on different arrangements have been completed. Nearly two years' operating experience has shown that an attendant is not required except for a few minutes daily for checking and inspection. The control system has given satisfactory service, the temperature being maintained within ±½ F of the desired value over long periods. Defrosting of the low temperature evaporators has not been necessary since the —70 F room has not, as yet, been called upon to accommodate tests involving considerable moisture transfer.

Lengthy observations have been made at temperatures of 0, —20, —40, and —60 F both with and without substantial heat loads. With F-12 in both the high and low temperature stages, using thermostatic expansion valves, and with no live load the lowest temperature attained was —85 F and this temperature was held continuously for a week. With a 1.4-kilowatt live load in the room the lowest temperature obtained was —72 F. The use of F-22 in the low temperature stage together with more complete flooding of the evaporators by means of a float valve and pump is expected to improve this performance.

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A LABORATORY SHAKER

By W. Harold White

A LABORATORY SHAKER[1]

By W. Harold White[2]

Abstract

A laboratory shaker is described that employs an eccentricless drive. The machine has sufficient flexibility in design to permit variable temperature and speed control. It has a capacity of two dozen 500-ml. flasks.

Introduction

Several years ago, a survey of existing laboratory shaking apparatus showed that an efficient machine of large capacity, suitable for certain types of food analysis, was commercially unobtainable. Special requirements, notably sufficient flexibility in design to permit variable temperature and speed control, prompted the designing of a machine embodying temperature maintenance at approximately 100° C., speed variation by means of cone pulleys, capacity for two dozen 500-ml. flasks, and a drive that does not depend upon eccentric motion. The present article has been prepared in response to requests for details of construction.

Details of Construction

The apparatus shown in Fig. 1 did not make use of the usual eccentric drive, since it was found that eccentric motion produced too abrupt a movement, particularly at high speeds. This obstacle was overcome by using the Eureka–Buhler 'eccentricless' drive,[*] consisting of two counterweighted steel wheels enclosed in drums, shown at B in Fig. 1, supported by a base-plate, L, and geared to rotate in opposite directions. The counterweights were so arranged that when they were in the vertical plane the centrifugal forces exerted by their rotation were equal and opposite, whereas when they were in the horizontal plane the forces were additive and produced a shaking motion.

Length of body travel is dependent upon the speed used and the weighting of the steel wheels at the factory; a travel of $\frac{1}{2}$ in. at the lowest speed was found to be most suitable for normal needs. As the speed of the shaker depends upon the speed of rotation of the steel wheels, variation may be obtained by changing the ratios on the cone pulleys at C and K, side view at D. Power was supplied by a 1/6 h-p. electric motor, C.

The apparatus may be mounted upon castors for mobility, as shown, or permanently established on wooden blocks.

The supporting framework was constructed from 1½ in. angle iron in the following dimensions: length, 3 ft.; width, 1 ft. 6 in.; height, 2 ft. 4 in.

The body of the shaker was constructed of 1-in. lumber, to form an open-top box 3 ft. 3 in. by 1 ft. 4 in. by 10½ in., outside dimensions. A copper steam chest, 1 in. in height and containing copper steam coils, was fitted into the bottom of the box, covered with a ¼ in. layer of felt to avoid damaging glassware, and overlaid with 1¼ in. insulation board, with holes of proper diameter to hold flasks of the desired size. These details are indicated in the cut-away section, *I*, in which the top layer represents the insulation board.
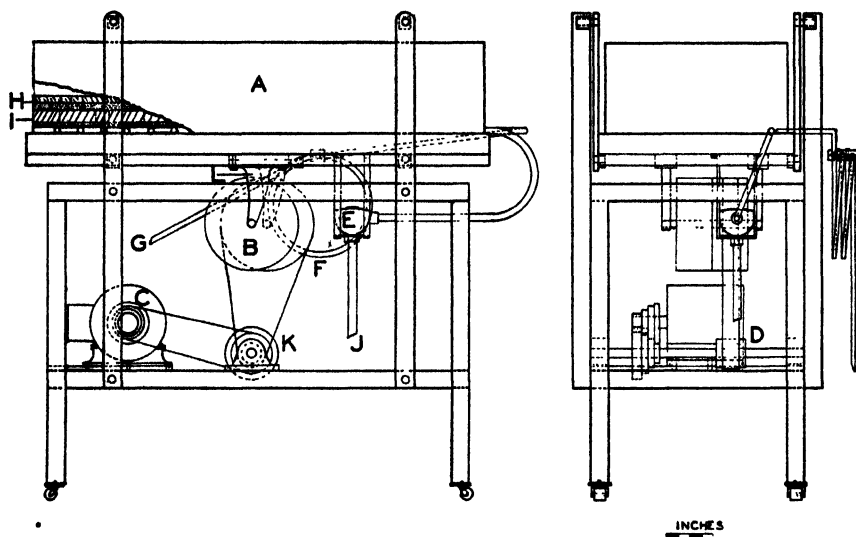
FIG. 1. *Side and end views of a laboratory shaking apparatus.*

A 7/16 in. plywood board cover, fitted with ½ in. felt insulation, is shown in section at *H*, resting on section *I*. This cover, with holes through which the flask-necks protrude, holds the flasks firmly in place.

The body of the shaker was mounted on four sets of bearings, two sets being shown in the end view in Fig. 1. This indicates a ½ in. flat brass bearing at the top of the supporting arm; at the bottom of the arm is a ½ in. rod, connected as an axle to the corresponding opposite arm.

Steam was introduced at *G*, representing the copper tubing inlet, passed through a shock-absorbing coil, *F*, which absorbed the motion produced when the shaker was in use, and thence to the metal steam chest. Returning steam was passed through a steam-trap, *E*, and any condensed water drained off through the outlet, *J*.

Thermostatic control may be installed for temperatures below 100° C., by circulating water from a temperature controlled reservoir in place of steam.

Chemical Disinfection and Corrosion Prevention

By H. L. A. Tarr

Pacific Fisheries Experimental Station
Vancouver, B.C.

ABSTRACT

The effectiveness of solutions of disinfectants in killing bacterial vegetative cells in a watery suspension of fish slime, on dry artificially contaminated wooden surfaces and in nutrient broth, as well as their ability to destroy bacterial endospores and mould spores, was investigated. The disinfectants studied included sodium hypochlorite, chloramine T, six cationic detergents, four chloro- or phenyl-phenols, phenol, formaldehyde, a commercial cresolic disinfectant and another containing sodium hypochlorite. The use of sodium nitrite as a corrosion inhibitor for three of the disinfectants when in contact with iron and copper was also investigated. The bearing of the results on the selection of disinfectants for use in the fishing industry is discussed.

There is still a pressing need for general improvement in the market quality of edible fish products, and one means of bringing this about is by adequate disinfection of fishing vessels, fish handling plants and retail premises.

In 1927 Bronkhorst (pp. 88-89), in connection with experiments on the control of bacterial reddening of salted fish, suggested that chemical disinfectants be used both on fishing vessels and in buildings in which the fish was prepared. He advised a thorough washing of all surfaces, preferably with hot water, and subsequent treatment with a chemical bactericide such as 2% sulphurous acid, "formalin" or potassium permanganate. He also believed that boats, and garments which workers used in handling the fish, should be sterilized. Later Bedford (1935) advocated that fishing vessels, after thorough cleansing with water, be disinfected by means of a 6% formalin solution applied with a high pressure spray. Gibbons (1935) stated that plants preparing salted fish should employ germicidal sprays such as a 2% formalin or "lye" solution in order to help control the red discolouration of the product. Hess (1940) gave general instructions for disinfection of plants which handle fish, and suggested that rooms might be sealed and sterilized by use of sulphur dioxide gas or formalin vapour, or that they could be sprayed with 2.5% formalin or lye solution. Hypochlorites have also been used quite extensively as disinfectants in the fishing industry. Dybwad (1937) recommended their use in canneries.

About two years ago experiments were undertaken at this Station in order to study a number of disinfectants, certain of which it was thought might prove suitable for use in the fishing industry, and the results of this work are described

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herewith. Brief preliminary accounts of this investigation have already been published (Tarr 1944, 1945).

Since Shewan (1945) found that dehydrated fish contain coccoid organisms potentially capable of producing food-poisoning, a known enterotoxin-producing coccus strain (*Staphylococcus aureus*) was included in the experiments.

EXPERIMENTAL

DISINFECTANTS

1. Sodium hypochlorite. One hundred and ten g. of H.T.H. powder (Mathieson), containing about 70% of calcium hypochlorite, and 60 g. of anhydrous sodium carbonate were dissolved separately in distilled water. The solutions were mixed, diluted to 1 litre, and filtered. The filtrate contained about 8% of sodium hypochlorite, had a pH of approximately 11.2 and was stable for months when stored in the dark at O°C.

2. Diversol. An alkaline powder containing 3.5% of sodium hypochlorite.

3. Roccal and R2L. Ten per cent solutions of alkyl dimethyl benzyl ammonium chloride.

4. Thoral. Ten per cent solution of 9-octadecenyl dimethyl ethyl ammonium bromide.

5. Cetavlon. A powder containing 75% of cetyl trimethyl ammonium bromide.

6. Emulsept and Norcalite. Ten per cent solutions of N-(acyl esters of colamino formyl methyl) pyridinium chloride.

7. E 607 M. The N-(myristic acid ester of colamino formyl methyl) pyridinium chloride.

(Nos. 3 to 7 inclusive cationic detergents.)

8. Chloramine T. Eastman Kodak Co.

9. Lysol. Lehn and Fink.

10. Dowicide A. Sodium ortho phenylphenate (Anon. 1941).

11. Dowicide C. Sodium chloro-2-phenylphenate (Anon. 1941).

12. Dowicide D. Sodium 2-chloro-4-phenylphenate (Anon. 1941).

13. Dowicide F. Sodium 2, 3, 4, 6-tetrochlorophenate (Anon. 1941).

14. Phenol. Merck's U.S.P.

15. Formaldehyde. Baker's C.P.

CULTURES

1. *Staphylococcus aureus*. ("Wood" strain, enterotoxin producer, from the University of British Columbia.)

2. *Micrococcus* sp. Culture 6, isolated from fish (Tarr 1939).

3. *Achromobacter* sp. Culture 9, isolated from fish (Tarr 1939).

4. *Achromobacter* sp. Culture 17, isolated from fish (Tarr 1939).

5. *Achromobacter* sp. Culture 22, isolated from fish (Tarr 1939).

6. *Bacillus* sp. Unclassified aerobic sporogenous *Bacillus* isolated from soil.

7. *Penicillium cyclopium* (from the National Regional Research Laboratory, Peoria, Ill.).

8. *Penicillium puberulum* (from the National Regional Research Laboratory, Peoria, Ill.).

PREPARATION OF BACTERIAL SUSPENSIONS

FISH SLIME SUSPENSION

A suspension containing a mixture of fish spoilage bacteria in a watery extract of fish slime was prepared as follows. A number of lemon soles (*Parophrys vetulus*) and herring (*Clupea pallasii*), which had been stored for a few days in ice, were washed in several litres of tap water so that a fairly cloudy suspension was obtained, from which the coarser particles were removed by filtering with suction through glass wool. The resulting suspension was stored for 1 day at about 5°C., after which it had a direct count (Tarr 1943) of 85×10^6 bacteria per ml. and a viable count (Tarr and Bailey 1939) of 4.1×10^6 colonies per ml. The apparent discrepancy in these counts was probably due to the fact that there were numerous clumps of bacteria in the suspension. A number of $\frac{1}{4}$-lb. (113-g.) tins were filled with the suspension, sealed, frozen in still air and stored at between about $-25°$ and $-30°C.$ until required. The frozen suspension was thawed immediately prior to use by immersing a can in water at 20 to 25°C. The following viable counts were obtained during the interval of storage throughout which the suspension was employed in experiments to be described: 3 days' storage, 740,000 colonies per ml.; 67 days' storage, 210,000 colonies per ml. The suspension contained 11.3 mg. of dry material in 5 ml.

MIXED BACTERIAL VEGETATIVE CELLS

Ten 9-cm. diameter petri dishes containing Bacto nutrient agar, pH 7, were inoculated with each of cultures 2, 3, 4 and 5 and were incubated for 3 days at 25°C. The growth was suspended in 2 l. of sterile distilled water and filtered with suction through sterile absorbent cotton wool. The suspension was then frozen and stored as in the case of suspension 1, and the following counts were obtained:

	Direct count, bacteria per ml. (Petroff-Hausser counting chamber)	Viable count, colonies per ml. (Tarr & Bailey 1939)
Unfrozen, immediately prior to freezing..	$7,000 \times 10^6$	$9,100 \times 10^6$
Thawed, after 1 day's storage............	$6,500 \times 10^6$	$8,700 \times 10^6$
Thawed, after 7 days' storage.........,...	---	$7,900 \times 10^6$
Thawed, after 373 days' storage.........	$7,000 \times 10^6$	$4,450 \times 10^6$

The suspension was used for disinfection experiments within 40 days of preparation.

ENDOSPORES

A number of petri dishes containing a 1.5% agar medium prepared from a 1:10 dilution of fish digest broth at pH 7.0 (Tarr 1942) as nutrient substrate were inoculated with culture 6 and incubated for 7 days at 30°C. The growth, which consisted mainly of endospores on this dilute medium, was suspended in sterile distilled water, filtered through absorbent cotton wool with suction and heated for 10 minutes at 80°C. The resulting suspension was frozen and stored in the usual manner, and the following counts were obtained:

	Direct count (spores per ml.)	Viable count (colonies per ml.)
Unfrozen, immediately prior to freezing..	290×10^6	110×10^6
Thawed, after 2 days' storage...........	330×10^6	120×10^6
Thawed, after 333 days' storage.........	280×10^6	85×10^6

The suspension was used between 300 and 330 days after preparation.

MOULD SPORES

Cultures 7 and 8 were grown for 14 days at 25°C. on each of ten 9-cm. petri dishes containing Bacto nutrient agar to which 5% glucose and 0.5% tartaric acid had been added (Henrici 1930). The mould spores were suspended in sterile distilled water, the suspension was filtered with suction through cotton wool and centrifuged, the precipitated spores were re-suspended in water, filtered again and frozen and stored in the usual manner. The following counts were obtained:

	Direct count (spores per ml.)	Viable count (colonies per ml.)
Immediately prior to freezing...........	48×10^6	29×10^6
Thawed, after 1 day's storage...........	49×10^6	27×10^6
Thawed, after 21 days' storage..........	49×10^6	21×10^6

In the experiments to be described, sterile tubes, pipettes and dilution water were used, and aseptic precautions were taken in making all dilutions of the germicides.

RESISTANCE TO DISINFECTANTS

BACTERIA IN FISH SLIME

The relative effectiveness of the different germicides in presence of fish slime was determined as follows.. Five-millilitre portions of appropriate serial dilutions of the germicide in 19 × 150-mm. tubes were placed in a water bath at 20°C., and a 5-ml. portion of thawed fish slime suspension (20°C.) was added to each tube and mixed thoroughly. After 10 minutes a 4-mm. loop was inserted into each tube and was withdrawn so that only a thin film of liquid adhered to it. This was transferred to a tube containing 10 ml. of Bacto nutrient broth (pH 7.0). Tubes thus inoculated were incubated for 7 days at 25°C. and the presence or

absence of growth was recorded. The final dilutions of germicide used in making these tests were as follows: 1:20 to 1:100 (intervals of 1:20), 1:100 to 1:1000 (intervals of 1:100), 1:1000 to 1:18,000 (intervals of 1:1000). In the results (table I), it will be seen that, under the conditions given, the cationic detergent germicides, with the exception of the one which was contaminated, were active in low concentrations. From about 0.02 to 0.1% of a germicide of this type would rapidly kill fish spoilage bacteria in the presence of a moderate amount of fish slime. Sodium hypochlorite and chloramine T showed about the same degree of germicidal activity as did the cationic detergents. The disinfecting power of Diversol corresponded very closely to its sodium hypochlorite content. The Dowicides and Lysol were somewhat less effective than the cationic detergents and hypochlorite, but were considerably better germicides than phenol or formaldehyde.

TABLE I. Comparative effectiveness of disinfectants against the natural mixed bacterial population of fish slime.

Disinfectant	Lowest concentration of disinfectant which prevented growth in sub-cultures
Roccal	1:10,000 } 1:9,500 1: 9,000
R 2 L	1:8,000 } 1:7,500 1:7,000
Norcalite	1:6,000
Thoral	1:5,000
Cetavlon	1:5,000
E 607 M	1:4,000
Emulsept*	All tubes down to 1:16,000 dilution showed growth.
Sodium hypochlorite	1: 9,000 } 1:9,500 1:10,000
Chloramine T	1:5,000
Diversol	1:300
Dowicide A	1:600
" C	1:2,000
" D	1:2,000
Lysol	1:500
Formaldehyde	1:100
Phenol	1:100

*Two different samples were examined and both were found to be contaminated with a spore-forming *Bacillus*. Viable counts showed that one sample contained 1.7 × 10⁶ and the other about 20 colonies per ml. of disinfectant. This contamination did not seriously impair the activity of this germicide toward certain bacteria, as is shown by experiments on disinfection of surfaces, which will be described.

ENDOSPORES

The technique was similar to that employed in the above experiment, except that thawed endospore suspension was employed in place of fish slime suspension, and a full 4-mm. loopful of medicated suspension was transferred to nutrient

broth as in the phenol coefficient test (Ruehl and Brewer 1931). The experiments extended over a 24-hour period. Two separate experiments were made; in one, undiluted endospore suspension was employed, and in the other the suspension was diluted 1:10 with water before use. The results (table II) show that, of the six disinfectants tested, only sodium hypochlorite rapidly killed the endospores. Formaldehyde killed the spores very slowly, and phenol and Dowicide D were inactive even after 24 hours. In 0.05% concentration Roccal and Norcalite did not inactivate the spores within 24 hours. The results with 0.5% of these compounds were shown in other experiments to be unreliable owing to the fact that usually sufficient germicide to prevent growth was carried over into the broth tubes used for sub-cultures. Very little difference was observed in the behaviour of the undiluted and diluted spore suspension under the conditions of the experiment.

TABLE II. Effect of disinfectants on bacterial endospores from culture 6. A = undiluted spore suspension; B = spore suspension diluted 1:10; + = visible growth; − = no visible growth.

Germicide	Concentration %	Exposure time (20°C.)																				
		5 min.		10 min.		20 min.		30 min.		1 hr.		2 hr.		3 hr.		4 hr		5 hr.		6 hr.		24 hr.	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Phenol	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Roccal	0.5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+	+	+	+	+	+	+	+	+·	+	+	+	+	+	+	+	+	+	+	+	+	+
Norcalite	0.5	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	−	−	−	+
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dowicide D	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Formaldehyde	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sodium hypochlorite	0.5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

MOULD SPORES

The experimental conditions were similar to those used for bacterial endospores, except that thawed undiluted and diluted (1:10) mould spore suspension was tested, transfers being made into Bacto nutrient broth containing 5% glucose and 0.5% tartaric acid. The results (table III) show that sodium hypochlorite killed mould spores more rapidly than did the other compounds tested, and that it was closely followed in effectiveness by Roccal and Thoral. Norcalite,

Dowicide D and formaldehyde killed mould spores rather slowly. The results obtained in the case of 0.5% Dowicide D were not very reliable, because tests indicated that a quantity of this disinfectant sufficient to inhibit growth of mould in the broth used for sub-culture was carried over by the 4-mm. loop employed.

TABLE III. Effect of disinfectants on mixed mould spores from cultures 7 and 8. A = undiluted spore suspension; B = spore suspension diluted 1:10, + = excellent growth with surface mycelial "mat"; +* = slight growth consisting of a clump of mycelia in the liquid and no surface growth; − = no growth.

Germicide	Concentration %	5 min.		10 min.		20 min.		30 min		1 hr.		2 hr.		3 hr.		4 hr.		5 hr.		6 hr.		24 hr	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Phenol	0.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+*
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Roccal	0.5	+*	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+*	+*	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Norcalite	0.5	+	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	+*	−	+	−	−	−	−	−	−
	0.05	+*	+*	+*	+*	+*	+*	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Dowicide D	0.5	−	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+*	+	+	+	+	+	+	+	+	+	+	+*	+	+	+*	+*	+*	−	−	−	−	−
Formaldehyde	0.5	+	+	+	+*	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+*	+	+*	+	+*	+	−	−
Sodium hypochlorite	0.5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	0.05	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Thoral	0.5		+*	−			−			−			−			−			−			−		
	0.05		+*		+*		−			−			−			−			−			−		

EFFECT OF ANTI-CORROSIVE

Roccal and formaldehyde were selected for this experiment because the former was shown to be very effective in killing mixed fish spoilage bacteria in the presence of fish slime, and because the latter has been used extensively as a germicide in the fishing industry. Sodium hypochlorite was not tested in this way because attempts to find a really satisfactory anti-corrosive for this compound have not as yet yielded very favourable results (*vide infra*). Phenol, Lysol and the Dowicides were not studied because they possess a distinct and rather penetrating odour which, although not detracting from their usefulness for certain disinfecting purposes, makes them appear rather unsuitable for use near fish or fish products. Sodium nitrite was selected for use as anti-corrosive for reasons which will be given later.

Cultures 1 and 5 were used as test organisms, and the standard F.D.A. phenol coefficient method (Ruehle and Brewer 1931) was followed, with the single exception that all cultures and subcultures were grown in Bacto nutrient broth (pH 7.0). Culture 1 was grown at 37° and culture 5 at 25°, the phenol coefficient being determined at 20°C. in the case of each culture. The results (table IV) show that sodium nitrite tends to increase the germicidal efficiency of both formaldehyde and Roccal, and that this is true for solutions in which nitrite and germicide were stored together for a considerable time. They also show that Roccal is a very much more effective germicide than is formaldehyde.

TABLE IV. Influence of sodium nitrite on the germicidal efficiency (on cultures 1 and 5) of Roccal and formaldehyde as judged by the phenol coefficient test.

Method of treatment of the germicide from which dilutions were made to determine the phenol coefficient.	Phenol coefficient	
	Culture 1	Culture 2
Untreated formaldehyde........	1.6	2.0
5% formaldehyde containing 1% sodium nitrite used directly after preparation.............	1.6	2.4
5% formaldehyde containing 1% sodium nitrite stored 57 days in a closed clear glass bottle at room temperature.....	1.6	——
Untreated Roccal.............	235	110
Solution containing 1% each of Roccal and sodium nitrite stored 120 days in a closed brown glass bottle at room temperature.	294	116

CONTAMINATED SURFACES

The method to be described was used in order to obtain some information regarding the ability of different concentrations of the germicides being studied to inactivate bacteria present on dry, heavily contaminated wooden surfaces.

Five 6.25-cm.² areas were ruled at 2.5-cm. intervals on the smooth planed surfaces of a number of 25 × 6.25 × 2-cm. fir boards. Prior to use, and between experiments, these were washed with hot trisodium phosphate solution, next with water, then with dilute acetic acid and again with water. They were sterilized by autoclaving and permitted to dry at room temperature. The boards were then placed on a level surface and 5 ml. of thawed mixed bacterial cell suspension were painted along a strip about 4 cm. wide so that all ruled areas on each surface were covered. The suspension was dried at room temperature with assistance of a fan. From 2 to 3 hours after contamination the surfaces were gently immersed for 5 seconds in a 200-ml. portion of either water or appropriately diluted germicide contained in a shallow glass dish. After immersion the boards were placed vertically and exposed to a fan so that the surfaces dried in about 5 minutes. The surfaces of the 5 ruled squares on each board were then washed thoroughly into 9 ml. of sterile water using a small cotton swab on an applicator, a suspension of roughly the same opacity being obtained in each case. The

numbers of viable bacteria in these suspensions were determined using the roll tube method.

The results (table V) will be seen to be very erratic, and this may be due

TABLE V. Decrease of viable bacteria on treating with different disinfectants
wood surfaces contaminated with a mixed bacterial suspension.

Disinfectant	Concentration %	Viable bacteria (colonies per ml. of washings)		Reduction by disinfectant in viable bacterial population (%)	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
Water (controls)		14×10^6			
		17×10^6			
		20×10^6			
		21×10^6			
		34×10^6		—	—
		44×10^6			
		Average =			
		25×10^6			
Sodium hypochlorite....	1.0	2,000	9	> 99.99	> 99.99
	0.1	11,000	62,000	> 99.99	99.8
	0.05	40,000	200,000	99.8	99.2
	0.01	370,000	230,000	98.6	98.9
	0.005	450,000	720,000	98.2	97.2
Roccal	1.0	0	0	100	100
	0.1	47,000	83,000	99.8	99.7
	0.05	430,000	1.1×10^6	98.1	95.6
	0.01	7.1×10^6	7.7×10^6	67.6	69.1
	0.005	11×10^6	14×10^6	56.0	44.0
Emulsept.......	1.0	0	0.5	100	> 99.99
	0.1	700,000	1.2×10^6	97.2	95.2
	0.05	7×10^6	1.8×10^6	72.0	92.8
	0.01	32×10^6	8.9×10^6	0	64.4
	0.005	28×10^6	15×10^6	0	40
E 607 M.....	1.0	0	2.0	100	> 99.99
	0.1	620,000	3.4×10^6	97.3	86.4
	0.05	7×10^6	24×10^6	72	4.0
	0.01	15×10^6	—	40	—
	0.005	15×10^6	—	40	—
Cetavlon........	1.0	1.5	1.0	> 99.99	> 99.99
	0.1	1.3×10^6	2.2×10^6	94.8	91.2
	0.05	3.2×10^6	12×10^6	87.2	52
	0.01	9.4×10^6	31×10^6	62.4	0
	0.005	10×10^6	14×10^6		

Disinfectant	Concentration %	Viable bacteria (colonies per ml. of washings)		Reduction by disinfectant in viable bacterial population (%)	
		Expt. 1	Expt. 2	Expt. 1	Expt. 2
Dowicide A.....	1.0	47,000	28,000	99.8	99.8
	0.1	14×10^6	27×10^6	44	0
	0.05	42×10^6	23×10^6	0	8
	0.01	35×10^6	—	0	—
	0.005	20×10^6	—	20	—
Dowicide C.....	1.0	74	3,000	> 99.9	> 99.9
	0.1	550,000	630,000	98.0	97.3
	0.05	4.2×10^6	3.9×10^6	83.0	84.4
	0.01	11×10^6	—	56.0	—
	0.005	20×10^6	—	20.0	—
Dowicide D.....	1.0	0	0	100	100
	0.1	1.2×10^6	3.1×10^6	95.2	87.6
	0.05	16×10^6	17×10^6	36.0	32.0
	0.01	17×10^6	—	32.0	—
	0.005	25×10^6	—	0	—
Dowicide F.....	1.0	19,000	3,500	> 99.9	> 99.9
	0.1	20×10^6	19×10^6	20.0	24.0
	0.05	17×10^6	29×10^6	32.0	0
	0.01	26×10^6	—	0	—
	0.005	24×10^6	—	4	—
Formaldehyde...	1.0	130	650	> 99.9	> 99.9
	0.1	3.3×10^6	17×10^6	86.9	32
	0.05	9.8×10^6	35×10^6	60.8	0
	0.01	20×10^6	44×10^6	20.0	0
	0.005	22×10^6	34×10^6	12.0	0
Phenol.........	1.0	147,000	200,000	99.6	99.2
	0.1	7.7×10^6	40×10^6	69.1	0
	0.05	14×10^6	13×10^6	44.0	48.0
	0.01	13×10^6	—	48.0	—
	0.005	16×10^6	—	36.0	—
Lysol..........	1.0	4,500	1,100	> 99.9	> 99.9
	0.1	5×10^6	16×10^6	80.0	36.0
	0.05	7.1×10^6	35×10^6	71.6	0
	0.01	14×10^6	—	44.0	—
	0.005	27×10^6	—	0	—
Diversol........	1.0	11,000	27,000	> 99.9	> 99.9
	0.1	920,000	1.5×10^6	96.3	94.0
	0.05	970,000	2.6×10^6	96.1	89.6
	0.01	3.6×10^6	6.0×10^6	85.6	76.0
	0.005	7.9×10^6	11×10^6	68.4	56.0

to a number of factors, such as uneven contact of germicide and bacteria on the surfaces, differences in time of contact between germicide and bacteria resulting from differences in rate of drying, variations in intensity of bacterial suspension obtained on swabbing the surfaces, variations in time from the making of the bacterial suspension to the completion of the viable counts during which the organisms were in some cases exposed to the germicide, and finally to a possible inhibitory effect of the germicide if carried over into the lower dilutions used in making the viable counts. The results can, therefore, give only a very rough idea of the effectiveness of the germicides in sterilizing surfaces. If an arbitrary standard of 99 to 100% kill be considered excellent, 90-99% good, and less than 90% relatively ineffective, then the following conclusions can be drawn.

Excellent: Sodium hypochlorite, 1.0, 0.1 and 0.05%; Roccal, 1.0 and 0.1%; 1% of all the other disinfectants studied.

Good: Sodium hypochlorite, 0.01 and 0.005%; Roccal, 0.05%; Emulsept, 0.1% (0.05% in one of two tests); E 607 M, 0.1% (in one of two tests); Cetavlon, 0.1%; Dowicide C, 0.1% (in one of two tests); Dowicide D, 0.1% (in one of two tests) and Diversol, 0.1 and 0.05%.

Relatively ineffective: 0.1% or less of Dowicide A, Dowicide F, formaldehyde, phenol and Lysol.

In general these results are rather similar to those obtained in the experiment using fish slime suspension, where sodium hypochlorite and Roccal appeared to be the most effective of the disinfectants studied, the other cationic detergents also proving very active. In both types of experiment, Dowicide A, formaldehyde and phenol were relatively ineffective.

PREVENTION OF CORROSION

On fishing boats and in fish plants, corrosion of metals, particularly iron, steel, copper and brass, must be controlled. Certain disinfectants are particularly corrosive (e.g. hypochlorites), while others are not. However, solutions of the latter type will cause corrosion of metal surfaces to the same extent as will water itself. For this reason a means of neutralizing the corrosive effect of watery solutions of disinfectants was sought. A number of inorganic salts such as chromates, hexametaphosphates and nitrites have been used to inhibit corrosion of certain metals. Of these, sodium nitrite was selected because its weak solutions are practically colourless and almost neutral. It has been used extensively as an anti-corrosive in alkaline washing powders used for cleaning surgical instruments and, more recently, for preventing corrosion in oil pipe lines (Wachter and Smith 1943) and of steel in water (Wachter 1945).

At first a corrosion inhibitor was sought for use with sodium hypochlorite solutions, since this disinfectant was shown by the above experiments to be the best all-round germicide of those studied. Tests showed that nitrite could not be used for this purpose, since it reacts with hypochlorite. Sodium hexametaphosphate (Calgon) and potassium chromate appeared to afford slight protection of iron surfaces in presence of hypochlorite, but neither could be considered as

really satisfactory. As a result of these findings, and for reasons already given in the section in which the effect of nitrite on the phenol coefficients of Roccal and formaldehyde was described, corrosion prevention was studied only in the case of three of the disinfectants, namely Roccal, Emulsept and formaldehyde.

The following qualitative method was used to determine the relative rate of corrosion of iron and copper in solutions of the disinfectants. Squares of polished black iron or copper with a surface area of 6.5-cm.^{2} and a thickness of approximately 0.05 cm. were placed in 9-cm.-diameter open petri dishes. Twenty millilitres of distilled water or germicide solution of the desired strength (with or without sodium nitrite as anti-corrosive) were added to each dish. The dishes were stored at room temperature, 20 ml. of water being added to each dish every 4 days to make up for evaporation in cases where the test extended over 4 days. Corrosion was determined qualitatively by recording the amount of visible rusting of iron, or green discolouration of copper, in the experimental solutions. The results (tables VI and VII) show that the slight corrosion of

TABLE VI. Prevention of corrosion of black iron in formaldehyde solutions.
The signs − to + + + + indicate relative degrees of corrosion of the metals.

Disinfectant	% NaNO₂ present	*Comparative corrosion after 4 days
2.5% formaldehyde (unbuffered) .	0	+ +
	0.01	−
	0 05	−
	0.1	−
	0.5	−
*2.5% formaldehyde (buffered) . . .	0.0	+ + + +
	0.01	+ + + +
	0.05	−
	0.1	−
	0.5	−
	1.0	−

*The unbuffered formaldehyde had a slightly acid reaction. The buffered solutions of formaldehyde were slightly alkaline in reaction, and were prepared from 5% formaldehyde solution containing 0.2% Na₂HPO₄.12H₂O.

black iron which occurs in formaldehyde solutions is entirely prevented by quite low concentrations of sodium nitrite. They also show that slightly alkaline solutions of formaldehyde corrode iron more readily than do untreated formaldehyde solutions which are faintly acidic in reaction, and that alkaline solutions require slightly higher concentrations of nitrite to prevent such corrosion. The experiments with Roccal indicated that its watery solutions are not appreciably more corrosive toward black iron than is water alone, and that the corrosion of iron which does occur in Roccal solutions can be very markedly inhibited by sodium nitrite when it is present in a concentration similar to that of the detergent itself. The anti-corrosive action of nitrite was more marked in

the case of Emulsept solutions than with Roccal. Noticeable inhibition of corrosion of copper was only evident in solutions containing 0.1 or 1.0% of detergent and sodium nitrite, and was more marked with Emulsept than with Roccal.

TABLE VII. Prevention of corrosion of black iron and copper in solutions of Roccal and Emulsept. The signs − to ++++ indicate relative degrees of corrosion of the metals.

Disinfectant %	NaNO$_2$ %	Comparative corrosion after days:					
		Iron			Copper		
		4	16	40	4	16	40
Roccal							
0	0	++++	++++	++++	−	+	+
0	0.01	−	−	−	+	+	+
0.01	0	++++	++++	++++	+	++	++
0.01	0.01	−	+	++++	+	++	++
0.1	0	++++	++++	++++	++	++	++
0.1	0.01	++	++++	++++	++	++	++
0.1	0.1	−	+	+	+	+	++
1.0	0	++++	++++	++++	+++	++++	++++
1.0	0.01	++++	++++	++++	+++	++++	++++
1.0	1.0	−	+	+	++	++++	++++
Emulsept							
0.01	0	+++	++++	++++	−	+	+
0.01	0.01	−	−	++++	−	+	+
0.1	0	++++	++++	++++	+	+	+
0.1	0.01	−	+++	++++	+	+	+
0.1	0.1	−	−	−	−	+	+
1.0	0	++++	++++	++++	++	++	++++
1.0	0.01	++++	++++	++++	++	++++	++++
1.0	1.0	−	−	−	−	+	+

DISCUSSION

Though the experiments which have been described above do not definitely show that any one of the disinfectants studied is invariably superior to another for use in the fishing industry, they do give a definite indication of those which may prove of value. Sodium hypochlorite was undoubtedly the best all-round disinfectant, because it not only destroyed bacterial vegetative cells very readily but also destroyed both bacterial endospores and mould spores. However, it is very corrosive toward iron, copper and, indeed, many other metals (Prucha 1930), and if employed should be washed away soon after use as Moulton (1929) suggests. The cationic detergents as a group killed bacterial vegetative cells when used in quite low concentrations, and certain of them also killed mould spores fairly rapidly, but they were practically ineffective against bacterial

endospores. Tests with two of these detergents showed that sodium nitrite could be added to their watery solutions as an anti-corrosive, and that in the case of one of them the added nitrite did not adversely affect its germicidal potency. For these reasons it would appear that cationic detergents offer definite possibilities as disinfectants for use in the fishing industry. The fact that they were not effective in killing bacterial endospores would not seriously detract from their value, because endospore-forming *Bacilli* are not very frequently encountered among the common fish spoilage bacteria, and the presence of a cationic detergent, with the possible exception of the samples of Emulsept used which contained viable endospores (table 1), would undoubtedly inhibit the germination of the spores and the growth of the resulting vegetative cells. Numerous references have appeared since 1935 in which preparation, properties, germicidal power and methods of analysis of cationic detergents have been described (Domagk 1935, 1938; Maier 1939; Krog and Marshall 1940; Baker, Harrison and Miller 1941; Auerbach 1943; Epstein, Harris and Katzman 1943; Epstein, Harris, Katzman and Epstein 1943; Hoogerheide 1945; Valko and Dubois 1945). It is unlikely that the Dowicides would prove of value for purposes of general disinfection because, although those studied proved quite good germicides, all possessed a rather penetrating odour which would render them somewhat unsuitable for use near fish or fish products. This is also true of Lysol and phenol. Formaldehyde was shown to be one of the least effective of the germicides studied, and although it has been used extensively in the fishing industry, and is compatible with sodium nitrite as anti-corrosive, its low potency would detract considerably from its germicidal value. Formaldehyde solutions are also unpleasant to use. Diversol, which is apparently considerably less corrosive than sodium hypochlorite (Prucha 1930), is a good disinfectant, its effectiveness being roughly proportional to its sodium hypochlorite content.

ACKNOWLEDGMENTS

SUMMARY

The effectiveness of a number of disinfectants in inactivating bacterial vegetative cells, bacterial endospores and mould spores was investigated. The following types of disinfectants were studied: sodium hypochlorite, cationic detergents, phenyl- or chloro-phenols, a cresol compound, phenol and formaldehyde. The methods of testing the disinfectants included treatment of mixed fish spoilage bacteria in the presence of a watery suspension of fish slime, disinfection of mixed dried bacterial cells on wood surfaces, employment of a standard phenol coefficient method and medication of aqueous suspensions of bacterial endospores or mould spores. The possibility of preventing the corrosion of iron and copper which might arise through use of disinfectants was also studied.

The results indicated that sodium hypochlorite was the best all-round disinfectant, since it killed bacterial vegetative cells very readily, and also killed mould spores and bacterial endospores. Diversol exerted a germicidal activity which was closely related to its sodium hypochlorite content. Attempts to prevent corrosion, which is marked in the presence of hypochlorite, were not successful. The cationic detergents destroyed bacterial vegetative cells quite readily, and two of them inactivated mould spores rapidly. None of those tested was an efficient killer of bacterial endospores. In the case of two of the cationic detergents tested, sodium nitrite was found to inhibit more or less strongly corrosion of iron and, to a smaller extent, of copper. In the case of one of these germicides the addition of sodium nitrite was found to have no adverse effect on its phenol coefficient. Though the phenolic germicides were quite effective bactericides, their solutions possess a marked odour and it is not known whether this would detract from their practical value. Both phenol and formaldehyde were poor disinfectants. The slight corrosion of iron which occurred in watery solutions of formaldehyde was abolished by addition of a small amount of sodium nitrite.

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Control of Rancidity in Fish Flesh

I. Chemical Antioxidants

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ABSTRACT

The development of rancidity in the naturally occurring fats of frozen fish was retarded by treatment with ascorbic acid (0.05%), ethyl, n-propyl, n-butyl or hexyl gallates (0.01 to 0.05%), and cysteine hydrochloride (0.05%). Ethanol ammonium gallate (0.02%), dodecyl thiodipropionate (0.05%), thiourea (0.05%), citric and tartaric acids (0.02%) were ineffective. The loss of surface red colour which occurred during storage of coho and red spring salmon was largely prevented by pre-treatment with 0.02% of ethyl or propyl gallate. Both NaCl and NaNO₂ acted as pro-oxidants in frozen fish. In unfrozen salmon flesh stored at 0° C. both ethyl gallate and NaNO₂ retarded fat oxidation and bacterial increase.

During recent years there has been a growing literature concerning the use of chemical antioxidants for retarding the development of rancidity in isolated animal and vegetable fats and oils, but less attention appears to have been paid to the possible direct application of such compounds to animal flesh as a means of controlling oxidation of the indigenous fats.

Bahr and Willie (1933) suggested that certain phenolic compounds such as hydroquinone, eugenol or α-naphthol be added to the salt or brine used in fish curing in order to retard "rusting." Oat flour has been found to inhibit the onset of rancidity in salted mackerel (Peters and Musher 1937; Lemon, Stansby and Swift 1937 a and b). However, Australian work (Anon. 1940, pp. 57-58) suggests that the antioxidant effect of oat flour when used on fish fillets is very weak, and is confined more to its action in slightly depressing the peroxide value of the fat than to causing an actual improvement in flavour of the stored fish. Since the publication of short preliminary articles dealing with the present work (Tarr 1944 a and b, 1945), Silver (1945) has shown that development of oxidative rancidity in brine-cured mackerel and herring can be retarded considerably if the cured fish is dipped in oil containing 0.2 per cent nordihydroguaiaretic acid (NDGA) prior to storage. Recently Smith, Brady and Comstock (1945) have shown that NDGA, gossypol and a mixture of d-isoascorbyl palmitate, soybean lecithin and tocopherols can be employed to retard the onset of rancidity in sliced bacon.

<center>MATERIALS AND METHODS</center>

ANTIOXIDANTS

Ethyl gallate. This compound was prepared by esterifying technical gallic acid (Beilstein 1927, p. 484). After two successive crystallizations from water and drying at 105° C. pale brown crystals of the anhydrous compound (M.P. 153-155°) were obtained. In one experiment the "Progallin A" of Nipa Laboratories was used.

n-Propyl gallate. Heyden Chemical Corporation. In one experiment "Progallin P" of Nipa Laboratories was used.

n-Butyl gallate. From Dr. C. H. Lea, Cambridge, England.

Hexyl gallate. Heyden Chemical Corporation.

Ethanol ammonium gallate. Silmo Chemical Corporation.

l-Ascorbic acid. Merck and Co., U.S.P. grade.

Cysteine hydrochloride. Eastman Kodak Co.

Thiourea. Eastman Kodak Co.

Dodecyl thiodipropionate. Du Pont de Nemours.

Sodium gallate. Aqueous solutions of Merck's U.S.P. gallic acid were adjusted to pH 6.0 with 1.0 N NaOH.

PREPARATION OF SAMPLES

Only strictly fresh fish were used and the antioxidants were incorporated as follows.

MINCED FLESH

Salmon (*Oncorhynchus* species) and black cod (*Anoplopoma fimbria*) were filleted and the fillets were skinned, while herring (*Clupea pallasii*) were scaled and gibbed. The flesh was comminuted with a Universal mincer having a plate with 4.5-mm. diameter holes. Antioxidants, NaCl or $NaNO_2$ were incorporated into the minced flesh by adding a 1% aqueous solution of the chemical slowly and beating the flesh in a bowl for from 2 to 3 minutes with Mixmaster beaters at speed 3. The 1% solutions of dodecyl thiodipropionate, hexyl gallate, n-butyl gallate, and n-propyl gallate were added while warm because these compounds are either sparingly soluble or almost insoluble in water at ordinary temperatures. In early experiments in which steam-extracted fat was used for determination of peroxide values, 200-g. samples of the minced flesh were wrapped in M.S.T. (moisture proof, heat sealing, transparent) cellophane and stored. However, for most of the experiments 25-g. samples were placed in 6-oz. (170-g.) wide mouth, screw-capped glass jars and then frozen and stored.

FILLETS AND SPLIT FISH

The fish was immersed for 1 minute at about 20° C. in a watery solution of the antioxidant or salt used, and drained for 5 minutes on a wire mesh screen prior to wrapping in M.S.T. cellophane, freezing and storing. In all cases a volume of solution sufficient to ensure that there would be little change in its composition during the treatment was employed.

FREEZING AND STORAGE

All samples were frozen in still air at the temperature at which they were to be stored. They were stored either in cabinets at -5, -10 or $-20°\pm 1°$ C., or in a large room the temperature of which varied from -24 to $-32°$ C. with a rough average of $-28°$ C.

DETECTION OF RANCIDITY

Lea (1938) has pointed out that no single available chemical test is likely to prove an absolute criterion of the degree of rancidity of a fat, and this is not surprising in view of the complex chemical changes which are involved during oxidation of unsaturated fats (Black 1945). However a quantitative determination of the amount of fat peroxides present in foods such as fresh mackerel (Stansby and Lemon 1941), frozen salmon fillets (Stansby and Harrison 1942), frozen herring (Banks 1937, 1938 and 1939), edible fats (Lea 1944b) and bacon (Smith *et al.* 1945) has proved a useful comparative index of the amount of fat oxidation which has occurred during storage, and in certain cases data thus obtained have closely paralleled those obtained by organoleptic tests. For this reason a measurement of the amount of fat peroxide present in the fish flesh has been used in these experiments as a comparative criterion of the degree of rancidity. In the case of both coho (*Oncorhynchus kisutch*) and red spring (*Oncorhynchus tshawytscha*) salmon the information derived from peroxide tests has been supplemented by that obtained from quantitative measurements of the disappearance of the surface red colour of samples during storage (*vide infra*).

In early experiments (see tables 1-6) peroxide values were determined on steam extracted fat as follows. Two-hundred-gram samples of minced flesh (defrosted where frozen) were placed in 1-l. conical flasks with 200 ml. of water, and a rapid stream of steam was run into the mixture for from 15 to 30 minutes until sufficient fat was obtained. The oily layer was decanted and roughly separated from the watery phase using a small separating funnel. The oil was placed in a small centrifuge tube, heated in boiling water, and centrifuged while hot. Peroxide values were obtained using 1-g. samples of the oil in the simplified method described by Lea (1938). This method was not found to be very satisfactory, partly because rather large samples of fish were required, but mainly because it could only be used with fish flesh which was quite rich in fat. For this reason the following method, which was similar in some respects to that described by Stansby and Lemon (1941) for determining peroxide values and free fatty acids in mackerel, was used in most of the experiments.

Twenty-five g. of minced flesh in a 6-oz. glass jar were mixed thoroughly with a like quantity of pure anhydrous sodium sulphate using a strong glass stirring rod with mushroomed end, and 50 ml. of pure chloroform were then incorporated. The jar was covered with a screw cap and kept in the dark for 15 to 30 minutes. The contents were mixed well, and filtered with suction after pouring on to a filter paper which had been moistened with chloroform and sucked on to the surface of a small Büchner funnel. Twenty ml. of the clear filtrate were placed in a 200-ml. conical flask, 30 ml. of pure glacial acetic acid

and 2 drops of saturated KI solution were added, and the flask was kept in a dark place for 10 minutes. Fifty ml. of water were added and the liberated iodine was titrated with 0.002 N $Na_2S_2O_3$ using approximately 2 ml. of acid starch indicator (Platner 1944). It was found that though the end point was not reached for several minutes (probably due to slow diffusion of the iodine into the watery layer), it was quite sharp and duplicates agreed closely. The amount of fat used for each determination was calculated from the weight of oil obtained after evaporating the chloroform from a 10-ml. sample of the above filtrate. The results are expressed as ml. of 0.002 N $Na_2S_2O_3$ per gram of fat. It was found that when ascorbic acid or ethyl gallate was added to samples of minced fish flesh in from 0.02 to 0.1% concentration, the peroxide values of the fats of such samples were not affected appreciably.

Since this work was commenced publications have appeared which show that peroxide determinations which are carried out in the presence of atmospheric oxygen yield rather higher results than do similar determinations when performed in presence of an inert gas (Lea 1945; Stuffins and Weatherall 1945). However, whether this would affect *comparative* results is apparently not known.

The initial peroxide values of the fat of fresh fish flesh were invariably zero.

Determination of Gallates

Fifteen grams of minced flesh containing ethyl or n-propyl gallate were mixed thoroughly with 45 g. of anhydrous Na_2SO_4, and 40 g. of the mixture were extracted for 1 hour with 100 ml. of ether in a Soxhlet apparatus. The ether was removed under reduced pressure, the residue extracted with 65 ml. of boiling water, and the amount of gallate present in the extract determined by the colorimetric method of Mattil and Filer (1944). The extract was suitably diluted where the gallate concentration in the flesh was over 0.01%, and as the solutions were slightly cloudy a colorimeter control sample was prepared from gallate-free fish flesh. It must be noted that the ethyl and n-propyl esters of gallic acid give in this method slightly more intense colours per mole of gallic acid than does gallic acid alone, and this must be taken into account when making determinations. Filer (private communication) has observed that with n-propyl gallate the development of colour is more rapid, the colour more intense, and the absorption curve of the coloured complex is different from that of pure gallic acid.

The following recoveries were obtained in an experiment in which given percentages of ethyl gallate were incorporated in white spring salmon flesh:—

Ethyl gallate added (%) ..	0.02	0.01	0.005	0.003	0.002	0.001
% recovery..............	100	94	104	113	90	90

This indicates that a recovery of about $100 \pm 6\%$ might be expected in flesh containing 0.01 to 0.02% ethyl gallate, but that in concentrations below 0.01% the method is less accurate.

The amount of ethyl gallate in spring salmon fillets approximately 12.5 × 7.5 × 2 cm. in size (240 to 270 g.) was determined after immersing them for different lengths of time at 20° C. in 0.5% ethyl gallate solution alone and in a

20% NaCl brine containing 0.5% ethyl gallate. The following results were obtained.

Immersing period (minutes)	0.5	1	5
% ethyl gallate in fillets immersed in:			
0.5% ethyl gallate	0.016	0.024	0.040
0.5% ethyl gallate + 20% NaCl	0.021	0.027	0.080

EXPERIMENTAL

Delaying Onset of Rancidity

UNFROZEN FISH

The effect of ethyl gallate and sodium nitrite on the keeping quality of minced white spring salmon flesh stored at 0° C. was investigated. Two 200-g. samples of the flesh were used for each treatment, peroxide values being made using steam extracted fat, and direct bacterial counts by a method previously described (Tarr 1943). The various treatments employed and the results obtained are given in table 1. It will be seen that 0.02% ethyl gallate prevented

TABLE I. Effect of ethyl gallate and sodium nitrite on bacterial increase and development of rancidity in unfrozen minced white spring salmon flesh stored at 0° C. Bacterial count is recorded as millions of bacteria per gram and P.V. = peroxide value determined on steam extracted fat.

Treatment	Bacterial count after days		P.V. after days	
	6	14	6	14
Untreated	95	670	1.1	1.0
0.02% ethyl gallate	1.8	280	0	0
0.02% NaNO$_2$	0.17	80	0.2	0.4
0.02% NaNO$_2$ + 0.02% ethyl gallate	0.05	4.8	0	0

the development of fat peroxides and also considerably retarded bacterial increase. Sodium nitrite (0.02%), the bacteriostatic action of which has been described in previous papers (Tarr 1941, 1942) inhibited bacterial growth markedly, and also prevented development of fat peroxides. Incorporation of both ethyl gallate and sodium nitrite caused greater inhibition of both bacterial growth and fat oxidation than did either of these compounds when used alone. It seems probable that the effect of nitrite in inhibiting formation of fat peroxides in unfrozen fish flesh, which is shown both in this and the following experiment, is linked up with its bacteriostatic action. Bacteria frequently accelerate fat hydrolysis and oxidation in meats (Jensen 1945). Sodium nitrite alone has been found to accelerate fat oxidation in frozen fish (*vide infra*).

Seventeen fillets approximately 12.5 × 7.5 × 2 cm. in size were cut from a 20-lb. (9.1-kg.), white spring salmon. One fillet was examined immediately,

the remainder being treated as recorded in table II (two fillets to each treatment) and then stored at 0° F. The results show that all treatments, listed as follows in order of preference, inhibited bacterial growth: NaNO₂; ethyl gallate, sodium gallate; ethyl gallate plus NaCl; sodium gallate; sodium chloride. Formation

TABLE II. Effect of ethyl gallate, sodium gallate, NaNO₂ and NaCl on bacterial increase and development of rancidity (peroxide value, P.V.) in unfrozen white spring salmon fillets stored at 0° C. P.V. determined on steam extracted fat.

Treatment	Bacterial count after days		P.V. after days	
	7	15	7	15
Untreated..................	125	380	0.4	0.95
Dipped in 0.5% ethyl gallate..................	1.7	410	0	0
" " 0.5% sodium gallate.............. ...	3.8	980	0	0
" " 0.5% NaNO₂...	0.34	2	0	0.15
" " 20% NaCl.................	6.2	540	1.3	0.95
" " 20% NaCl + 0.5% ethyl gallate..	0.11	210	0	0
" " 20% NaCl + 0.5% sodium gallate.......	1.7	640	0	0.1
" " 20% NaCl + 0.5% NaNO₂.......	0.13	2.3	0	1.15

of fat peroxides during the short storage period was entirely prevented by ethyl gallate, sodium gallate and ethyl gallate plus sodium chloride, and was retarded by NaNO₂, NaNO₂ plus NaCl and sodium gallate plus NaCl. Fat oxidation was accelerated by treating fillets with NaCl alone.

In connection with the results recorded in the above experiments it is of interest that bacterial growth has also been found to be markedly retarded by treatment of fish flesh with n-propyl gallate.

FROZEN FISH

Herring. Herring which had been stored frozen in glazed blocks for several months were defrosted, and 24 split fish were subjected to each of the treatments outlined in table III. The minced flesh from 12 fish was used for each peroxide value test. The results of this experiment (table III) show that ethyl gallate proved a good antioxidant, especially in the higher concentrations employed,

TABLE III. Effect of ethyl gallate and sodium gallate on development of rancidity in frozen split herring stored at −5° C. Peroxide value (P.V.) determined on steam extracted fat and initially = 2.0.

Treatment	P.V. after days	
	46	117
Untreated..	17.6	35.7
Dipped in 1% sodium gallate.............................	13.0	25.6
" " 0.5% sodium gallate........	17.6	25.6
" " 0.1% " "	17.0	26.4
" " 1.0% ethyl gallate.............................	2.2	3.1
" " 0.5% " "	2.0	5.7
" " 0.1% " "	6.5	10.4

but that sodium gallate was practically inactive in this respect. Further experiments were made, minced flesh of fresh herring, with and without various antioxidants, being frozen and stored in glass jars at either −10 or −20° C. The results show that ethyl gallate (tables III to VI), n-propyl gallate (tables IV and V), n-butyl gallate (table IV), hexyl gallate (table VI), ascorbic acid and

TABLE IV. Effect of gallic acid esters and dodecyl thiodipropionate on development of rancidity in minced herring flesh stored at −10° C. Peroxide value (P.V.) determined on steam extracted fat after 107 days' storage and thereafter on chloroform extracted fat.

Treatment	P.V. after days		
	107	176	231
Untreated.......	30.1	78.0	148
0.025% ethyl gallate.	16.1	18.3	47.5
0.05% ethyl gallate.	6.7	10.9	25.5
0.025% n-propyl gallate.	14.9	23.5	52.5
0.05% n-propyl gallate.	7.4	10.4	29.1
0.025% n-butyl gallate..	15.7	19.9	48.8
0.05% n-butyl gallate..	9.0	13.0	22.9
0.05% dodecyl thiodipropionate..	26.4	54.5	75.5

cysteine hydrochloride (table V) all caused a marked inhibition in the rate of development of rancidity. On the other hand dodecyl thiodipropionate (tables IV and V), thiourea (table V) and ethanol ammonium gallate (table VI) had either little or no protective action, or, in the case of thiourea, exhibited a prooxidant effect. Fat oxidation in split fresh herring was retarded by dipping them in 0.2% solutions of either ethyl gallate or ethanol ammonium gallate

TABLE V. Effect of six different antioxidants used in 0.05% concentration on development of rancidity in minced herring flesh stored at −20°C., as determined by peroxide values.

Days stored	Untreated	Ethyl gallate	n-Propyl gallate	Ascorbic acid	Thiourea	Cysteine hydrochloride	Dodecyl thiodipropionate
57	1.9	0.1	0.5	0.5	1.9	0.5	1.5
100	3.3	1.1	1.6	1.1	15.7	2.4	4.3
150	5.5	2.0	2.9	2.6	42.5	3.7	5.2
310	32.9	8.0	9.9	16.0	97.8	24.4	31.1

TABLE VI. Effect of ethyl gallate, hexyl gallate and ethanol ammonium gallate in 0.02% concentration on development of rancidity in minced herring flesh stored at −10° C., as determined by peroxide values.

Days stored	Untreated	Ethyl gallate	Hexyl gallate	Ethanol ammonium gallate
15	6.9	1.4	2 3	6.9
35	11.1	8.3	11.0	13.8
70	45.3	30.5	57.1	77.9
91	61.3	56.0	56.5	71.1

(table VII). Since ethanol ammonium gallate is very soluble in water it is assumed that a much greater concentration was attained in the split herring than the 0.02% which was without effect in the experiment with minced herring flesh (table VI).

TABLE VII. Effect of ethyl gallate and ethanol ammonium gallate on the development of rancidity in split herring stored at −20° C., as determined by peroxide values.

Days stored	Untreated	Dipped in 0.2% ethyl gallate	Dipped in 0.2% ethanol ammonium gallate
16	0.5	0	0.3
49	1.9	0.4	0
98	5.1	2.2	4.0

Spring Salmon. Minced flesh of fresh red spring salmon, with and without added antioxidants, was frozen and stored in glass jars at different temperatures. It was found that ascorbic acid, ethyl gallate, n-propyl gallate and cysteine hydrochloride all retarded development of rancidity (tables VIII and IX), dodecyl thiodipropionate had no protective value and thiourea at first retarded and subsequently accelerated fat oxidation. In these experiments ascorbic acid and the gallates strongly retarded bleaching of the red astacin pigments of salmon flesh, while cysteine hydrochloride did not have this effect. The rate of fat oxidation bore an inverse relationship to the storage temperature (table IX).

TABLE VIII. Effect of six different antioxidants used in 0.05% concentration on development of rancidity in red spring salmon flesh stored at −20° C., as determined by peroxide values.

Days stored	Untreated	Ethyl gallate	n-Propyl gallate	Ascorbic acid	Thiourea	Cysteine hydrochloride	Dodecyl thiodipropionate
55	1.0	0	0	0	0.3	0.2	1.1
103	2.2	0.2	0.3	0.4	0.2	0.7	1.9
176	5.6	2.0	1.5	0.2	5.0	1.3	4.6
260	9.9	4.7	4.3	0.4	18.4	4.0	11.1
355	14.3	10.9	8.0	0.6	64.4	7.3	12.3

TABLE IX. Effect of ethyl and n-propyl gallate on development of rancidity in minced red spring salmon flesh stored at −10, −20 and −28° C., as determined by peroxide values.

Days stored	−10° C.			−20° C.			−28° C.		
	Untreated	0.02% ethyl gallate	0.02% n-propyl gallate	Untreated	0.02% ethyl gallate	0.02% n-propyl gallate	Untreated	0.02% ethyl gallate	0.02% n-propyl gallate
32	3.1	0.4	0.2	0.5	0	0	...	...	...
64	8.1	0.5	1.5	0.2	0	0	0.5	0.2	0.2
96	21.8	7.9	7.1	2.7	0.6	0.3	0.6	0	0
140	15.6	5.2	4.8	2.8	0.4	0.4	1.0	0.1	0
212	25.4	6.6	6.4	6.8	2.2	2.4	2.7	0.8	0.9
361	33.7	7.3	7.3	13.7	5.2	6.8	4.1	1.5	1.8

The influence of heating (cooking) untreated and treated minced red spring salmon flesh, which had been stored at $-20°$ C., on the peroxide value of extracted fat was determined. Samples were "cooked" by placing the jars of defrosted fish flesh in warm water, covering them, and exposing them for 10 minutes to boiling water. The types of treatment and results of analysis of uncooked and cooked samples are given in table X. The results showed that lower peroxide values were obtained in the heated flesh irrespective of the method of pre-treatment, and that while ethyl gallate exerted marked antioxidant activity, $NaNO_2$ acted as a pro-oxidant.

TABLE X. Effect of cooking on the peroxide values of the fat of untreated and treated red spring salmon flesh stored at $-20°$ C.

Days stored	Untreated		0.02% ethyl gallate		0.02% $NaNO_2$	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
0	0	0	0	0	0	0
39	2.0	0.9	0.1	0	5.0	2.5
79	5.5	2.7	1.1	0.6	9.2	3.8
128	12.4	6.7	3.8	3.0	15.9	6.3
186	17.0	13.5	6.6	4.9	15.6	7.6
266	20.3	10.8	13.3	8.3	16.5	13.6

Twenty-four fillets approximately 12.5 × 7.5 × 2 cm. in size were cut from two 8 to 9-pound (3.6 to 4.1-kg.) red spring salmon, dipped in 10% NaCl solution, and drained. Eleven fillets were left untreated, the remainder being dipped in 0.25% ethyl gallate solution. Analysis of the pooled minced flesh of two of the treated fillets showed that it contained 0.0096% ethyl gallate. Treated and untreated fillets were stored at both -10 and $-28°$ C. The results of the experiment (table XI) show that rancidity developed much more rapidly at the higher storage temperature, and that at both storage temperatures ethyl gallate delayed fat oxidation considerably. The rather irregular increase in peroxide values obtained during storage was probably because fat oxidation does not

TABLE XI. Effect of ethyl gallate on development of rancidity in red spring salmon fillets stored at -10 and $-28°$ C., as determined by peroxide values (P.V.).

Days stored	$-10°$ C.		$-28°$ C.	
	Controls	Ethyl gallate	Controls	Ethyl gallate
* 84	4.3	1.5	0.2	0
*116	5.4	2.1	1.3	0.4
*149	17.4	5.5	1.4	0.9
†196	13.3	5.0	5.1	0.3
†226	26.8	10.2	1.4	0.5
†317	....		4.3	0

*P.V. determined on steam extracted fat.
†P.V. determined on chloroform extracted fat.

proceed at the same rate in different fish or parts of the same fish, due to such possible differences as variations in fat content, area of exposed fat and amount of fat oxidizing enzyme (*vide infra*) present. Also the treated fillets may have had somewhat different concentrates of ethyl gallate.

Pink Salmon. The minced flesh from two 4-lb. (1.8-kg.) pink salmon (*Oncorhynchus gorbuscha*) was treated in different ways and then samples were stored at −10, −20 and −28° C. The methods of treatment employed and the experimental results are given in table XII. The results showed that the

TABLE XII. Effect of ethyl gallate, n-propyl gallate, ascorbic acid, sodium nitrite and sodium chloride on development of rancidity in minced pink salmon flesh.

Treatment	Peroxide value after days											
	−10° C.				−20° C.				−28° C.			
	45	90	176	215	45	90	176	215	45	90	176	215
Untreated	5.8	12.4	33.8	38.8	0.8	2.3	10.8	15.1	0.2	1.0	2.0	3.5
0.02% ethyl gallate	0.3	1.7	12.5	13.4	0.2	0.7	2.0	4.2	0	0.2	0.6	0.8
0.02% n-propyl gallate					0.1	0.8	3.2	5.0				
1.0% NaCl					5.1	12.7	24.5	33.4				
1.0% NaCl + 0.02% ethyl gallate					0.7	2.3	6.7	11.8				
1.0% NaCl + 0.02% n-propyl gallate					0.5	2.5	14.3	17.1				
1.0% NaCl + 0.02% NaNO₂					6.5	13.3	29.7	31.8				
0.02% NaNO₂					1.6	7.1	20.5	21.4				
0.05% ascorbic acid					0.2	0.5	0.3	1.2				

rate of fat oxidation was considerably reduced by lowering the storage temperature, and that ethyl gallate inhibited development of rancidity to about the same extent at each of the three storage temperatures. At −20° C. ethyl and n-propyl gallate protected the fish to about the same extent, and both considerably delayed the onset of rancidity in fish flesh treated with NaCl, which itself accelerated fat oxidation. Sodium nitrite exerted a pro-oxidation effect, and a mixture of NaNO₂ and NaCl promoted fat oxidation to a somewhat greater extent than did either alone. Ascorbic acid was a more effective antioxidant than were ethyl and n-propyl gallates.

Fillets were prepared from five 4-lb. (1.8-kg.) pink salmon, each fillet being cut in two pieces of approximately equal size. Four of the resulting small fillets (one from each fish) were dipped into solutions the composition of which are given in table XIII. The amount of gallate present was determined in one fillet from each treatment, and the remainder were stored at −10° C. The results (table XIII) show that ethyl and n-propyl gallate (average content 0.0143%) when thus incorporated into the flesh retarded fat oxidation considerably, and that initial buffering to pH 6.2 of the gallate solutions used did not appreciably affect their antioxidant power.

Chum Salmon. The compounds listed in table XIV were incorporated into the minced flesh of a 7.5-lb. (3.4-kg.) chum salmon (*Oncorhynchus keta*), and

the resulting samples were stored at −10° C. It will be seen from the results given in table XIV that ethyl gallate considerably retarded the onset of rancidity, and that ascorbic acid was not very effective in the concentration used. Neither citric nor tartaric acid retarded fat oxidation.

TABLE XIII. Effect of ethyl and n-propyl gallate on development of rancidity in pink salmon fillets stored at −10° C., as determined by peroxide values.

Days stored	Untreated fillets	*Dipped in 0.5% †ethyl gallate	*Dipped in 0.5% †ethyl gallate + 0.05 M sodium phosphate buffer pH 6.2	*Dipped in 0.5% †n-propyl gallate	*Dipped in 0.5% †n-propyl gallate + 0.05 M sodium phosphate buffer pH 6.2
43	3.5	0.5	0.5	0.2	0.3
67	8.6	2.5	3.0	2.5	1.4
97	10.4	5.1	4.5	3.3	2.8

*Final pH of buffered solutions about 6.3; initial pH of unbuffered gallate solutions about 4.5, and final pH about 6.2.

†Gallate content of fillets: Untreated fillets, none; dipped in 0.5% ethyl gallate, 0.014%; dipped in buffered 0.5% ethyl gallate, 0.0137%; dipped in 0.5% propyl gallate, 0.0104%; dipped in buffered 0.5% propyl gallate, 0.019% (Average = 0.0143%).

TABLE XIV. Effect of ethyl gallate, ascorbic acid, tartaric acid and citric acid on development of rancidity in minced chum salmon flesh stored at −10° C., as determined by peroxide values.

Days stored	Untreated	0.02% ethyl gallate*	0.02% ascorbic acid	0.02% citric acid	0.02% tartaric acid
52	27.9	7.6	9.15	29.5	21.6
101	31.3	14.8	25.2	39.2	38.0
132	58.0	23.6	47.7	74.5	60.3

* "Progallin A."

TABLE XV. Effect of n-propyl gallate, ascorbic acid and sodium chloride on development of rancidity in chum salmon fillets stored at −10° C., as determined by peroxide values.

Days stored	Untreated fillets	Dipped in 0.5% n-propyl gallate	Dipped in 20% NaCl	Dipped in 20% NaCl containing 0.5% n-propyl gallate*	Dipped in 0.5% ascorbic acid (pH 6.0)	Dipped in 0.5% ascorbic acid dissolved in 20% NaCl (pH 6.0)**
53	9.4	1.5	20.5	2.0	14.0	19.0
103	20.0	4.9	124.0	14.0	24.2	31.0
165	50.3	11.1	105.0	25.0	31.0	42.0

*The NaCl caused the gallate to crystallize from the solution at 20° C., so that this solution had to be used at 40° C.

**The pH of the ascorbic acid solutions was adjusted to 6.0 with 1.0 N. NaOH immediately prior to use.

Eighteen fillets approximately 12.5 × 7.5 × 2 cm. in size were cut from three 8-lb. (3.6-kg.) chum salmon. Three fillets were subjected to each of the treat-

ments recorded in table XV, and all were then stored at −10° C. The results (table XV) showed that NaCl treatment accelerated the onset of rancidity, and that n-propyl gallate treatment inhibited it. Immersion in ascorbic acid solutions alone did not hinder fat oxidation, but fillets which were dipped in NaCl solutions containing ascorbic acid did not become rancid as rapidly as those treated in NaCl solutions alone. It is suggested that NaCl facilitated the penetration of ascorbic acid into the flesh. Recent experiments (unpublished) have shown that immersion of fillets for at least 5 minutes in 1% ascorbic acid solution is necessary if a concentration of approximately 0.05% of the acid in the flesh is to be attained.

TABLE XVI. Effect of ethyl gallate, n-propyl gallate and sodium nitrite on development of rancidity in minced coho salmon flesh stored at −10 and −20° C., as determined by peroxide values.

Days stored	−10° C.				−20° C.			
	Untreated	0.02% ethyl gallate	*0.02% n-propyl gallate	0.02% sodium nitrite	Untreated	0.02% ethyl gallate	*0.02% n-propyl gallate	0.02% sodium nitrite
42	6.2	0.9	0.8	16.2	....	....	...	..
64	12.4	6.5	4.5	14.7	2.8	0.6	0.4	8.9
109	31.2	10.6	5.8	31.4	6.3	1.8	1.1	11.6
196	43.5	20.9	14.9	42.0	24.8	11.2	10.1	27.2
263	57.3	14.5	13.3	46.5	32.1	21.4	19.4	23.2
289	..	.	....	....	22.6	34.2	23.1	42.1

*Progallin P.

TABLE XVII. Effect of sodium chloride, ethyl gallate and ascorbic acid on development of rancidity in flesh of black cod stored at −10° C., as determined by peroxide values.

Days stored	Untreated	0.02% ethyl gallate	1.0% NaCl	0.02% ethyl gallate + 1.0% NaCl	0.05% ascorbic acid
35	0.7	0.5	0.5	0.4	0.1
69	2.1	2.5	3.0	3.2	0.1
108	4.9	4.9	5.7	8.1	0.3
158	3.3	5.1	6.8	6.3	0.4

Coho Salmon. The flesh from four 4-lb. (1.8-kg.) coho salmon ("bluebacks") was minced, and the flesh subjected to different treatments (table XVI). The results obtained on storing untreated and treated samples at −10 and −20° C. are recorded in table XVI. It will be seen that fat oxidation proceeded very much more rapidly at −10 than at −20° C. At both these temperatures 0.02% of ethyl or n-propyl gallate considerably delayed the onset of rancidity while 0.02% NaNO₂ exerted a pro-oxidant effect.

Black Cod. Minced flesh was prepared from a 16-lb. (7.25-kg.) black cod (*Anaplopoma fimbria*), sodium chloride, ethyl gallate and ascorbic acid were incorporated, and the samples stored at −10° C. The methods of treatment

and results are recorded in table XVII. It will be seen that fat oxidation was only slightly accelerated by sodium chloride, and that ethyl gallate did not retard this oxidation in either untreated or NaCl-treated flesh. Ascorbic acid strongly retarded development of rancidity.

STABILIZATION OF COLOUR

The development of rancidity in red salmon is accompanied by a superficial bleaching of the red astacin pigments, which is particularly evident in regions where the flesh is exposed to atmospheric oxygen. Heating the flesh also has a marked bleaching effect on colour. The following experiment was made in order to determine the effect of ethyl and n-propyl gallate and of $NaNO_2$ on the colour of salmon flesh after heating it, and also during prolonged storage in the frozen state. Aluminum dishes 5.5 cm. in diameter and 1.3 cm. deep were packed with minced coho or red spring salmon flesh which had received the treatments recorded in tables XVIII and XIX. Some of the samples were placed in covered

TABLE XVIII. Effect of ethyl gallate, n-propyl gallate and $NaNO_2$ on the red and yellow colour* of the surface of raw and heated coho salmon flesh, and of the raw flesh after freezing and storage at $-20°$ C.

Treatment	Raw		Heated		Period stored at $-20°$ C. (days)									
					64		141		219		303		303†	
	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y
Untreated......	12	6	6	3.5	5.5	3.5	5.5	3	3.5	3	2.5	3	11	6
0.02% ethyl gallate........	12	6	6	3.5	6	3.5	6.0	3	6	3	6	3.5	11	6
0.02% n-propyl gallate........	11.5	6	6	3.5	6	3.5	6.5	3	6.5	3	6.5	3.5	11	6
0.02% $NaNO_2$...	11.5	6	6.5	3.5	6.5	4.0	4.5	3	3	3	2.5	3	11.5	6
0.02% ethyl gallate + 0.002% $NaNO_2$	11.5	6	6.5	3.5	6.5	4.0	6.5	3	6	3	5.5	3.5	10.5	6

*In tables XVIII and XIX the colour is given in red (R) and yellow (Y) Lovibond units.
†The figures in this column are for the surface obtained after cutting the samples in half and thawing the exposed area. They show that the colour loss was largely superficial.

pyrex glass petri dishes, steamed for 20 minutes at $100°$ C., and then cooled. Others were stored in similar containers at $-20°$ C. The surface colour of the raw and heated flesh, and of the stored samples, was measured in red and yellow Lovibond units using an Armstrong colorimeter (Charnley 1936). The results (tables XVIII and XIX) show that, in general, the different treatments affected coho and red spring salmon similarly. Heating occasioned considerable loss in both red and yellow colour of untreated flesh. The gallates exerted no important effect on the colour of either raw or heated flesh. Sodium nitrite treatment alone, or in combination with ethyl gallate, slightly lowered the red colour of raw flesh but intensified that of heated flesh. Freezing caused an initial marked

TABLE XIX. Effect of ethyl gallate, n-propyl gallate and NaNO₁ on the red and yellow colour of the surface of raw and heated red spring salmon flesh, and of the raw flesh after freezing and storage at −20° C.

Treatment	Raw		Heated		Period stored at −20° C. (days)									
					62		131		209		294		294*	
	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y
Untreated.......	11	6.5	6	3.5	5	3	4	2.5	3.5	2.5	3	3	5.5	4
0.02% ethyl gallate........	11	6.5	6	3.5	6	3	6	2.5	5.5	2.5	5.5	3.5	6.5	4
0.02% n-propyl gallate........	11	6.5	6	3.5	6	3	6	2.5	5.5	2.5	6	4	7	4
0.02% NaNO₂...	10.5	6.5	6.5	3.5	5	3	3.5	2	2.5	2.5	2.5	3	9	4
0.02% ethyl gallate + 0.002% NaNO₂	10.5	6.5	6.5	3.5	6	3.5	4	2	5	2.5	5	3.5	6.5	4

*The figures in this column are for the surface obtained after cutting the samples in half and thawing the exposed area. The results in this case differ from those obtained with coho salmon (table XVIII) in that there was considerable loss of red colour in the sub-surface flesh, though this loss was less marked in treated samples than it was in the control.

loss in both red and yellow colour, and this may have been due to surface drying or some other effect, because freshly cut thawed surfaces of the coho salmon after 303 days' storage at −20° C. gave red and yellow colour readings nearly as great as those obtained initially in the raw flesh. In the stored frozen samples there was a marked progressive diminution in the red colour, and this loss was more rapid in flesh treated with NaNO₂. Both ethyl and n-propyl gallates prevented, or very strongly retarded, loss of red colour in the stored samples, and ethyl gallate prevented loss in colour in flesh treated with 0.002% NaNO₂. The yellow colour of the cold-stored fish samples differed slightly, and at that rather irregularly, in the different samples, and there is no definite indication that the small observed fluctuations were due to any of the treatments used. That the loss of red colour in the frozen coho salmon was largely superficial was apparent from the colour readings obtained in the thawed sub-surface flesh. In the red spring salmon this difference was not as marked, for the red pigments of the sub-surface flesh had been noticeably bleached.

DISCUSSION

Banks (1937) provided experimental evidence which proved that the rancidity developing in cold-stored herring was largely enzymic in origin, and that the lipoxidase enzyme concerned was activated by pure sodium chloride. He also showed that oxidation of the fat in frozen herring proceeded fairly slowly at −28° C., and much more rapidly at higher storage temperatures (1938). A preliminary storage at fairly high temperatures (e.g. −2.5 or −10° C.) was found to partially inactivate the lipoxidase enzyme so that on subsequent storage at lower temperatures fat oxidation in herring thus treated was greatly retarded (Banks 1939). It would seem probable that this inactivation may be linked up

with denaturation of a protein group of the enzyme concerned at high storage temperatures.

The results obtained in the present work with frozen coho, red spring and pink salmon, showed that rancidity developed much more rapidly at high than at low storage temperatures. It was also found that the onset of rancidity in cold-stored herring, black cod and salmon was hastened by treatment with pure sodium chloride. These findings confirm and extend those of Banks. However, in this connection it must be noted that recent work (Tarr 1946, and unpublished) has shown that sodium chloride treatment does not invariably accelerate fat peroxide formation, especially with autumn-caught fish, and also that the rate of peroxide formation may vary enormously with different lots of the same variety of fish stored at the same temperature. There are indications that fat oxidation proceeds very rapidly in frozen flesh prepared from "feedy" herring, and it is possible that lipoxidase enzymes from the feed gain access to the herring flesh.

Sodium nitrite in 0.02% concentration invariably accelerated the onset of rancidity in frozen fish, but had the reverse action in unfrozen fish, possibly due to its bacteriostatic action. Lea (1936) found that at pH values below 5.0, 0.03% of sodium nitrite was a powerful pro-oxidant, the activity of which was not affected by antioxidants which he studied. It would seem that at the usual pH of fresh fish flesh (6.0 to 6.8) nitrites are also able to act as pro-oxidants.

The experiments described showed that, except in the case of black cod, fairly low concentrations (e.g. 0.02 or 0.05%) of the lower alkyl esters of gallic acid (particularly ethyl and n-propyl gallates) markedly retarded fat oxidation in frozen fish, and largely prevented bleaching of the red astacin pigments of salmon. When used in similar (per cent) concentration the above esters appeared to be about equally effective. On the other hand the salt, ethanol ammonium gallate, was ineffective in 0.02% concentration. Whether or not the gallates only act directly as antioxidants, or also indirectly by partially inactivating the lipoxidase enzyme, is not certain. Gallic acid and its ester, the use of which as antioxidants in foods has been patented by Sabalitschka and Boehm (1941, 1942), have been found to be excellent antioxidants for whole dried milk (Findlay, Smith and Lea 1945), dried meat (Lea 1944a) and certain edible fats (Golumbic and Mattill 1942; Lea 1944b; Higgins and Black 1944). Moreover, the lower esters, when used in 0.02% concentration, do not affect the taste of treated products (Lea 1944a and b). The toxicity of ethyl or n-propyl gallates toward white mice and rats is extremely low (Boehm and Williams 1943; Hilditch 1944).

Ascorbic acid in 0.05% concentration was found to be a very good antioxidant for minced flesh of herring, red spring salmon, pink salmon and black cod. In one experiment using chum salmon 0.02% of this compound retarded fat oxidation only very slightly. Experiments which are still in progress indicate that immersion of fillets for about 5 minutes in ice-cold ascorbic acid solution (e.g. 1%) is required in order to attain a sufficiently high concentration to retard development of rancidity in treated flesh effectively. Ascorbic acid has been used successfully as an antioxidant for whole dried powdered milk (Findlay et al. 1945), for fats (Mattil, Filer and Longenecker 1944) and for certain other

foods (Gray and Stone 1939). Younga, Esselen and Fellers (1944) have shown that d-isoascórbic acid also possesses marked antioxidant activity.

The results obtained with thiourea, which has been shown to retard development of rancidity of fats in aqueous systems (György, Stiller and Williamson 1943) were not encouraging. In one experiment it inhibited the onset of rancidity slightly during early stages of storage, but subsequently acted as pro-oxidant, while in a second experiment it merely acted as pro-oxidant. Williamson (1944), working with powdered milk, found that thiourea was quite a good antioxidant during the early stages of storage but eventually exerted marked pro-oxidant activity in many cases. Cysteine hydrochloride markedly retarded fat oxidation, but it did not prevent bleaching of the red pigments of salmon flesh. Dodeycyl thiodipropionate did not protect the fats of fish flesh against oxidation.

There is little doubt that development of rancidity in stored frozen fish is one of the principal factors leading to its deterioration. Undesirable changes such as the appearance of a "salt fish" flavour in white fish of low fat content, of definite organoleptic rancidity in the flesh of fatty fish, and of bleaching of natural red pigments and "rusting" of exuded fat must all be included under this heading. The rate of development of well-defined organoleptic rancidity in cold-stored fish varies with different species, and is probably influenced by a number of factors such as changes in fat content, degree of unsaturation of the fats, and amount of lipoxidase enzyme in the tissue. Though a quantitative determination of the peroxide value of the fat of frozen fish might give a definite indication that the fish is or is not rancid, it is unlikely that such a test can ever be used to divide fish flesh into grades. Thus organoleptic rancidity has first been detected in kippers when peroxide values as different as 2.2 and 14.3 have been obtained (Banks and Reay 1938; Banks, Cutting and Reay 1939). In the present work it was found that in certain samples, and especially in those stored at $-10°$ C., there was initially a rapid increase in the peroxide value, but that this frequently became either stationary or only rose slowly. This may have been due to inability of oxygen to penetrate to the sub-surface flesh, or to a breakdown of the fat peroxides, or to both. In any case it indicates one of the difficulties which might arise in attempts to apply a determination of the peroxide value of the fats as a criterion of the quality of frozen fish.

SUMMARY

The effect of a number of chemical antioxidants, alone or in conjunction with NaCl and $NaNO_2$, on the development of rancidity in the indigenous fat of salmon, herring and black cod flesh stored at different temperatures was investigated.

At $0°$ C. ethyl gallate, sodium gallate and $NaNO_2$ retarded both fat oxidation and bacterial increase, while NaCl acted as a pro-oxidant.

In frozen flesh rancidity developed very slowly at $-28°$ C., and somewhat more rapidly at $-20°$ C.; while storage for a month or less at $-10°$ C. usually led to marked fat oxidation. Ethyl, n-propyl, n-butyl and hexyl gallates when used in from about 0.01 to 0.05% concentration in different experiments considerably retarded the onset of rancidity, while sodium gallate and ethanol

ammonium gallate (0.02%) were ineffective. Ascorbic acid in 0.05% concentration in minced flesh proved a very effective antioxidant, but in one experiment 0.02% was relatively ineffective. Cysteine hydrochloride retarded fat oxidation but did not prevent bleaching of the red astacin pigments of salmon flesh. Dodecyl thiodipropionate, thiourea and citric and tartaric acid were not found suitable as antioxidants for fish flesh.

The red and yellow pigments of coho and red spring salmon flesh faded considerably on heating and, superficially, on freezing. The marked superficial fading of the red colour of frozen coho and red spring salmon flesh during storage was largely prevented by pre-treatment with 0.02% of ethyl or n-propyl gallate, but was accelerated by $NaNO_2$ treatment.

Both NaCl and $NaNO_2$ accelerated oxidation of fat in frozen fish flesh.

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DRIED WHOLE EGG POWDER

XXVII. FURTHER OBSERVATIONS ON THE OCCURRENCE OF *SALMONELLA* ORGANISMS IN CANADIAN POWDER[1]

By N. E. Gibbons[2]

Abstract

During 1945, *Salmonella* organisms (other than *S. pullorum*) were isolated from 112 of 400 samples (28.0%) of Canadian dried egg powder. Fourteen types were found: *S. oranienburg, S. typhimurium, S. thompson, S. montevideo, S. newington, S. bareilly, S. manhattan, S. potsdam, S. anatum, S. newport, S. paratyphi B* (tartrate positive), *S. bredeney, S. tennessee,* and *S. selandia* (listed in order of prevalence). *S. pullorum* was isolated from 17 samples. In the majority of samples the most probable number of organisms was less than one per gram. No *Salmonella* organisms were found on the shells of 12,276 commercial eggs; *Salmonella* were isolated from the shells of eight of 24 eggs and later from the meats of two of 144 eggs from infected flocks.

Introduction

Previous studies indicated that *Salmonella* types were present in Canadian dried egg powder produced during 1943 (6). In November, 1944, it was suggested that this survey be resumed. Consequently all plain dried egg powders produced from the latter part of November, 1944, to the end of December, 1945, were examined. By this time four plants were producing dried sugar–egg powder (1, 11) and as most of the others were preparing to produce this type of powder, the survey was discontinued. Since dried sugar–egg powder is used exclusively for baking purposes there should be no health hazard (3, 7).

The present paper summarizes the findings of this study. The result of some incidental work on the source of these organisms is also reported.

Materials and Methods

Composite samples of powder from each carlot of powder produced were sent to a central control laboratory where they were thoroughly mixed under aseptic conditions for bacteriological and chemical analyses (12). Approximately 60-gm. portions of this mixed material were placed in sterile bottles, stored at approximately 40° F., and collected weekly for examination. Only Grade *A* powders were examined.

The methods used have been reported previously (6). Quadruplicate 5 gm. portions were examined throughout the study. From July on, both Difco SS and bismuth sulphite agars were used. Starting in September, whenever the first examination (total 20 gm.) was negative, the remainder of

the powder (usually 30 to 40 gm.) was cultured. Preliminary identification was made by means of O-sera and fermentation reactions. All cultures identified as *Salmonella* were typed by Dr. P. R. Edwards of the Salmonella Centre, Lexington, Ky.

The most probable number of organisms per gram of powder was determined for a number of samples (6). It was observed that even when three of four 5-gm. lots were positive the M.P.N. was usually less than one per gram. Hence in the latter part of the work, estimates were made only if all the quadruplicate portions were positive.

Results

From the latter part of November, 1944, until Dec. 31, 1945, samples from 400 carlots of powder were examined and *Salmonella* types (other than *S. pullorum*) isolated from 112 (28%). This is five times the incidence reported previously (6). *S. pullorum* was found in 17 samples.

The distribution by plants is shown in Table I. As before, there was no relation between the finding of *Salmonella* organisms and the plate count or total microscopic count of individual carlots, as given in the official reports of the Special Products Board. Rather there were indications of a contamination carried over in the plant. On several occasions, two, three, or even four carlots in succession would contain the same type.

TABLE I

DISTRIBUTION BY PLANTS OF SAMPLES OF POWDER POSITIVE FOR *Salmonella* TYPES OTHER THAN *S. pullorum*, ARRANGED IN ORDER OF PREVALENCE

Plant	Carlots examined	Lots positive	Percentage positive	Average bacterial content thousands/gm.	
				Plate	Microscopic
Western					
I	59	40	67.8	110	2550
H	79	24	30.4	76	720
G	35	9	25.7	74	670
A	13	3	23.1	39	2000
B	62	11	17.7	16	520
K	4	0	0	37	320
	252	87	34.5		
Eastern					
F	27	6	22.2	120	580
C	63	11	17.4	275	1400
E	50	8	16.0	50	2900
D	8	0	0	65	18,350
	148	25	16.8		
Total	400	112	28.0		

If average plate counts over the whole period are taken there is some relation with the incidence of *Salmonella* in the western plants (Table I). There is also some relation with direct microscopic counts. This relation is not as apparent with the eastern plants. Plant *D* operated practically entirely with frozen melange and pasteurized all liquid egg.

There is no apparent relation between the type of drier and the incidence of *Salmonella* organisms. Plants *I* and *H* are very similar in design and the powder is removed rapidly. Plants *B* and *E* are also similar in design and operation, and have about the same incidence. It is also true that the eastern plants *F* and *C* operate at higher temperatures than the western plants. Plant *C* began to use a preheater in March, 1945, and no *Salmonella* organisms were isolated during the period April to December. This was the only preheater used in Canada during the period under review and the results confirm the findings of Schneider (15). However, it is our opinion that the number of contaminated eggs is greater in Western Canada and that this in relation to plant operation determines the incidence and survival of *Salmonella* organisms. This opinion has not been substantiated, as very little experimental work has been done.

The distribution with time is shown in Table II. In this series a few more lots were positive in the fall and winter months than in the summer. This is the opposite of the previous series (6). On the over-all basis, the percentage of positive samples was remarkably constant throughout the year. The few positives in March and April are believed due to laboratory conditions.

TABLE II

DISTRIBUTION OF *Salmonella*-POSITIVE SAMPLES BY MONTHS

Month	From western plants			From eastern plants			Total		
	Carlots examined	Lots positive	Percentage positive	Carlots examined	Lots positive	Percentage positive	Carlots examined	Lots positive	Percentage positive
Nov.	5	1	20 0	3	2	66 6	8	3	37 5
Dec.	19	7	36.8	13	6	46 1	32	13	40 6
Jan.	25	9	36 0	14	4	28 6	39	13	33 3
Feb.	24	9	37 5	15	4	26 6	39	13	33 3
March	25	2	8.0	17	1	5 8	42	3	7 1
April	22	3	13.6	13	0	0	35	3	8 6
May	21	7	33.3	14	2	14 3	35	9	25 7
June	24	8	33 3	10	0	0	34	8	23 5
July	19	4	21 0	11	0	0	30	4	13 3
Aug.	15	8 (10)[1]	53.3 (66 6)	11	0	0	26	8 (10)	30 8 (38 4)
Sept.	14	5 (7)	35.7 (50 0)	7	0	0	21	5 (7)	23 8 (33 3)
Oct.	13	5 (7)	38 4 (53 8)	7	1 (2)	14 3 (28 6)	20	6 (9)	30 0 (45 0)
Nov.	18	6 (8)	33 3 (44 4)	7	1	14 3	25	7 (9)	28 0 (36 0)
Dec.	8	5	62.5	6	3	50 0	14	8	57 1
	252	79 (87)	31.3 (34.5)	148	24 (25)	16.2 (16.8)	400	103 (112)	25.7 (28 0)

[1] *Figures given in parentheses include isolations from larger amounts of powder (see text).*

In Table II is also shown the increase in positive samples due to the examination of the remaining 30 to 40 gm. of powder when the first examination proved negative. From September to December, 85 carlots were examined and *Salmonella* isolated from 36, nine of these or 25% being negative on the first test but positive in the larger sample. These increases made little difference in the order of incidence noted in Table I. Only one additional lot was found positive from the eastern plants (*E*). Four additional isolations were made from powder from Plant *I*, and two each from Plants *H* and *G*. These findings serve to emphasize the spotty distribution of these organisms in egg powder (6). It is also evident that, because of the small samples used in this investigation, the actual percentage incidence is higher than that reported here.

In the eastern plants, the percentage of carlots positive for *Salmonella* was greater in powders prepared from frozen egg than in those from shell or shell and frozen egg mixed (Table III). In one western plant (*H*) the percentage incidence increased when frozen egg was used, but in the others the type of egg used made little difference. In Plant *G* two of the four positive lots from shell egg were obtained with the larger samples of powder. If these are not considered there is little change in the percentage of positive samples from the different types of egg.

TABLE III

Carlots of powder, containing *Salmonella* organisms, produced from shell eggs, mixtures of shell and frozen egg, and frozen egg

Plant	Shell			Shell and frozen			Frozen		
	No. carlots	No. positive	Per-centage positive	No. carlots	No. positive	Per-centage positive	No. carlots	No. positive	Per-centage positive
Western									
A	4	1	25	1	—	—	8	2	25
B	16	3	18	33	5	15	13	3	23
G	10	4	40	4	1	25	21	4	19
H	13	2	15	38	8	21	28	14	50
I	22	14	63	30	21	70	7	5	71
K	—	—	—	—	—	—	4	—	—
	65	24	37	106	35	33	81	28	34
Eastern									
C	7	—	—	53	11	20	3	—	—
D	1	—	—	1	—	—	6	—	—
E	18	—	—	25	4	8	7	4	57
F	7	2	29	15	2	13	5	2	40
	33	2	6	94	17	18	21	6	29
Total	98	26	26	200	52	26	102	34	33

Fifteen types of *Salmonella* were found. These are listed in their order of incidence in Table IV. Most of the types found previously (6) were again encountered. *S. oranienburg* was the most common type, although 27 of the 45 strains were isolated from the powder produced by one plant. The three

TABLE IV

TYPES OF *Salmonella* ISOLATED AND THEIR DISTRIBUTION

Type	No. of samples positive in:		Total
	252 western lots	148 eastern lots	
S. oranienburg	45	2	47
S. typhimurium	14	5	19
S. pullorum	12	5	17
S. thompson	9	5	14
S. montevideo	0	9	9
S. newington	7	1	8
S. bareilly	4	1	5
S. manhattan	4	—	4
S. potsdam	3	—	3
S. anatum	2	1	3
S. newport	—	1	1
S. paratyphi B	1	—	1
S. bredeney	1	—	1
S. tennessee	1	—	1
S. selandia	1	—	1
			134

strains of *S. potsdam* were from the same plant (*H*) from which it was previously isolated (6). Of the new types, *S. montevideo* was found only in powder from eastern driers; eight of the nine isolations were from one drying plant. The strain of *S. paratyphi B* was tartrate positive; this is apparently a rare type in fowl. As far as can be determined, *S. selandia* has not been reported previously from fowl or eggs. It might be noted that one culture of *Aerobacter aerogenes* having antigens VI and VII was encountered from Plant *I*. Recently, several reports listing types of *Salmonella* isolated from dried egg have appeared elsewhere (14, 15, 18).

In eight lots of powder two types of *Salmonella* were encountered. In all but one instance the types were of different groups since it was impossible to distinguish types in the same group with the sera available for the preliminary screening. The following pairs were obtained: *S. oranienburg* and *S. bareilly*; *S. oranienburg* and *S. newington*; *S. typhimurium* and *S. manhattan*; *S. typhimurium* and *S. bareilly*; *S. newington* and *S. bareilly*. *S. pullorum* was also encountered, along with *S. oranienburg* and with *S. bredeney*. All of the above were isolated from the product of one drier (*I*). *S. pullorum* and *S. typhimurium* were isolated from a sample from Plant *H*.

Of the 17 strains of *S. pullorum* isolated, 14 were found only on bismuth sulphite agar plates. The superiority of this medium over SS agar for *S. pullorum* was also noted in isolations from chicks. This has recently been

reported by others (16). Schneider (15) claims that selenite F enrichment is superior to tetrathionate broth for the isolation of *S. pullorum*, although it is not stated whether equally good results were obtained with both the SS and desoxycholate citrate agars used. In the present study, of 51 isolations of types other than *S. pullorum*, 36 were isolated on both media, nine from SS agar only, and six from bismuth sulphite agar only. When organisms of the same group were found on both media it was assumed they were of the same type.

Of the 17 strains of *S. pullorum* isolated, 12 were checked for the 'X' variant (2, 19) by means of partially absorbed sera. Three were classed as the variant, five as intermediates, and four as the normal strain.

Estimâtes of the most probable number of *Salmonella* present were made on 54 lots. During most of the period, estimates were made only on samples in which three or four of the quadruplicate lots were positive. Of the 54 samples checked, in all but four the M.P.N. was less than one per gram. In four samples the M.P.N. was 1.3, 2, 3, and 9.4 per gram.

During June and July, samples from 12 carlots of dried sugar–egg powder from Plant *F*, six from Plant *A*, and three from Plant *B* were examined. *S. pullorum* was isolated from one lot from Plant *A*. No other *Salmonella* types were encountered in this product.

Source of *Salmonella* Infection

There is a growing literature to support the suggestion made previously (6) that in hens' eggs the *Salmonella* organisms, other than *S. pullorum*, come from fecal contamination on the shells, as has been noted by many authors with other types of fowl. Watt (17) isolated *S. montevideo* from the meats of eggs responsible for an outbreak, and in an examination of over 5000 hen eggs recovered *S. cholerasuis* and *S. derby* from a few. He apparently did not find *Salmonella* on the shells. Although the evidence was not very definite, the outbreak reported by Crowe (5) was probably due to infected meats. On the other hand, it has been shown that the shells of eggs laid by hens carrying *Salmonella* organisms are at times contaminated with these organisms (8). Gordon and Buxton (10) also indicate that in an outbreak in a hatchery the organisms were probably carried by fecal contamination. Chase and Wright (4) failed to isolate any *Salmonella* types, other than *S. pullorum*, from the meats of 2000 and the exterior of 400 eggs. A recent contribution (16) gives ample evidence that more dirty eggs carry *Salmonella* organisms than do clean eggs. Our attempts to trace the source of these organisms are reported below.

From November, 1944, to August, 1946, eggs were received from six points across Canada at monthly intervals for another study (13). Five dozen each of Grades *A* large, *A* medium, *A* pullet, *B*, and *C* were received each time and broken out for drying. The shells of each lot were collected in sterile beakers, packed loosely, and covered with tetrathionate broth. In all, the

shells of 195 lots or 975 dozen eggs were examined. Samples (approximately 100 ml.) of the broken out egg were also examined. *S. pullorum* was isolated from two samples of melange and one lot of shell. No other *Salmonella* organisms were encountered.

In addition, the shells and contents of some 48 dozen other eggs of various grades have been examined with negative results. This represents a total of 12,276 eggs from various parts of Canada. However, this is a very small sample when it is considered that between 120,000 and 180,000 dozen eggs go into one carlot of powder.

In an infected flock one might expect different results. When a number of chicks from a Saskatchewan hatchery were found to be infected with *S. bareilly*, the eggs from which the chicks were hatched were traced back to two farms, and in May, 1945, a dozen eggs from each flock was obtained. Both shells and egg meats were examined by a technique already described (9). The untreated egg is placed between ring clamps, which are kept painted with tincture of iodine, and the meat is removed as previously indicated. The shell is then broken up using two pairs of flamed forceps and put into tetrathionate broth. The small amounts of iodine carried over are neutralized by the excess thiosulphate in the broth. *S. bareilly* was isolated from the shells of two of the eggs from one farm (*L*) and of six of the eggs from the other (*M*). No organisms were found in the meats. In August six dozen eggs were obtained from each of these farms. All from one lot (*L*) were negative, but *S. newington* was found in the meats of two eggs from the other farm (*M*). No *Salmonella* organisms were recovered from the shells of these eggs.

In Plant *I* in May, 1946, the shell and contents of 40 dirty eggs were examined as well as swabs of the shells of 89 dirty eggs. No *Salmonella* was obtained. Samples of melange, shell drip, etc., were taken at various times but only one isolation was made; *S. pullorum* was found on an emulsifier screen. Three samples collected on one day in July from the by-pass to the high pressure pump and one from the holding vat were positive for *S. oranienburg*.

At Plant *H*, 52 dirty eggs were examined, with negative results. Five lots of shells from at least three dozen eggs each were collected at the breaking tables and cultured but no *Salmonella* was found. However, *Salmonella* types (*S. bareilly*, *S. oranienburg*, and *S. thompson*) were recovered in 7 out of 12 samples of the albumen draining from the barrels of egg shells. During the same period, isolations were made from 4 of 20 samples of melange taken at various points in the plant (*S. potsdam*, *S. bareilly*, *S. thompson*). It cannot be said whether these organisms came from the inside or outside of the eggs, although the greater incidence in the shell drippings would point to the latter.

It would seem therefore that where flocks are known to be carriers or if large enough samples of commercial eggs are examined, *Salmonella* organisms may be recovered from the eggs. The evidence presented is still insufficient to say that it is due solely to fecal contamination.

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Freezing Rates of Fruits and Vegetables at Various Air Velocities

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THE effect of freezing rates on the quality of frozen fruits and vegetables has been under discussion since the inception of the frozen food industry. It is well known that a rapid transition past the freezing point results in small ice crystals. This small ice crystal formation tends to maintain intact the normal structure of biological material. With large ice crystal formation cellular and fibrous structures are liable to be torn and ruptured.

Previous studies with asparagus made by Dr. Mary MacArthur of the (Canadian) Horticultural Division have shown that even with the most rapid commercial freezing rates some tissue rupture occurs. Furthermore, there was evidence to show that subsequent holding temperatures have an effect on ultimate crystal size. Thus tissue rupture continues while in the frozen state. Furthermore, tissue rupture increases at a greater rate at 0 F than at sub-zero temperatures of about —20 F.

The frozen food processor wants to know the following facts concerning freezing rates:

1. What is the relation between rate of freezing and quality from the standpoint of flavor, texture and appearance?

2. If, from the standpoint of quality or any other reason, fast freezing rates are required, what is going to be the comparative cost of freezing various products at different rates?

It is intended that the information derived from the following experiments will, in part at least, answer these questions. Individual circumstances change with both time and location, necessitating some modification in the application of fundamental principles.

The method for determining freezing rates in the following work consisted of following temperature trends at various locations within the product. These trends were established by temperature readings made at intervals determined by copper constantan thermocouples in conjunction with a potentiometer with a 32 F junction setup. The freezing points determined by this method, were checked with actual observations in identical samples (except for absence of thermocouple).

Only one size and type of package was used throughout these experiments. This was a heavily waxed, cylindrical cardboard carton 3½ in. diameter and 4 in.

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deep (inside measurements). A slip-on cover with overlap (⅝ in.) was used. These packages have a recess of ¼ in. at both top and bottom. In all cases the cartons were filled as full as possible without damage to the product.

It was almost impossible to eliminate all the head-space. This was particularly true with asparagus; with this product the stalks were cut to 4 in. so that the tips came in contact with the cover.

Where vegetables were packed in brine a 2 per cent (by weight) sodium chloride solution was used. In the
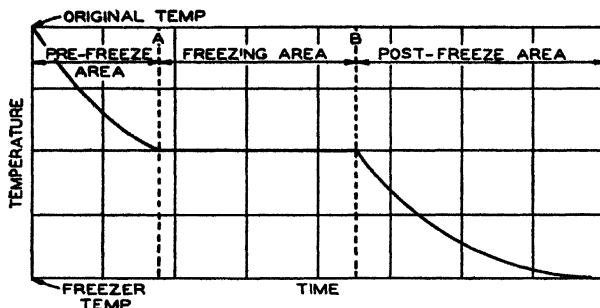
Fig. 1. Typical freezing curve. Trend of product temperatures when placed in freezer.

syrup packs, for fruit, a 45 per cent (by weight) sugar solution was used. With all products the actual weight of fruit or vegetables was the same in both the wet and dry packs, the solution being merely used to fill the interstices. The average net weights of the various products were as follows:

Asparagus—14 oz vegetables plus 6 oz brine
Peaches—15 oz fruit plus 5 oz syrup
Raspberries—9.8 oz fruit plus 9 oz syrup
Strawberries—12.5 oz berries plus 9.4 oz syrup

The static (still air) freeze was accomplished at the outset by placing the packages on a sheet of corrugated paper on the concrete floor of the storage room. The freezing rate curves of these samples showed considerable variance so latterly these samples were placed in the air tunnel with the cover removed and the fan shut off. This tunnel was of frame construction, 36 in. long by 23 in. wide by 12 in. high, with a dividing shelf in the center. A fan was located at one end to blow air from the storage room (temperature controlled) longitudinally through the tunnel. The packages were so placed that uniform exposure to the air stream was effected. Baffles were used to provide uniformity in the air stream.

In all samples, temperature trends revealed a typical freezing curve with certain modifications. This typical curve is shown in Figure 1. It will be seen from this chart that when the product is placed in the freezer its temperature immediately drops until the freezing point is reached. At the freezing point of the product (about 22 to 26 F depending on product) the temperature becomes constant until it (the product) is frozen. Following freezing the temperature curve assumes approximately the same trend as in the pre-
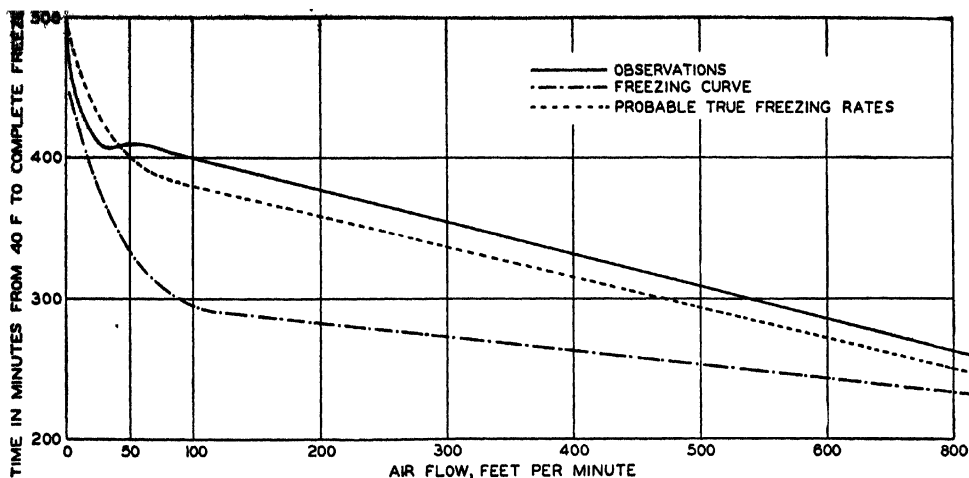
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Fig. 2. Freezing rates of strawberry dry pack at 0 F in a freezing tunnel employing various wind velocities.

freeze area until its fall slows off at it approaches the freezer temperature.

By actual observation it was found that the point of complete freezing did not coincide with point B (Fig. 1). This was found to be caused by two factors. (No correlation was established at point A between observed and suggested start of freezing.)

Thermocouples Affect Readings

Factor 1—The more thermocouples that were placed in the product the greater was the spread between point B and the observed freezing point. It was concluded therefore that the high conductivity of heat through the thermocouple wires caused an increase in the freezing rate. This was substantiated when cartons with and without thermocouples were compared. Even one thermocouple in a pint package increased the freezing rate. Unfortunately, the increase varied with the method of freezing.

Factor 2—It was not always possible to forecast which would be the last portion of the product to freeze. When the thermocouple was located ½ in. from the last freezing point in a pint package, it indicated as much as a 30 per cent reduction in the time spent at the freezing point. In other words the center of a package may remain at the freezing point while the outer portion of the package is well below the freezing point. This is particularly true under very rapid freezing conditions.

From a practical standpoint these factors may be inconsequential. Where absolute data are concerned, it can readily be seen that actual observation is much more reliable than temperature trends established by the use of thermocouples. Determining actual freezing points by observation also has its drawbacks. Entering the freezer to remove packages for observation disturbs the temperatures. Furthermore, even by observation it is quite difficult to judge when the last portion to freeze is actually frozen. Observations at close intervals require a large amount of material. In this work it is felt that comparable information is of more value than absolute data.

Variations with Temperature and Air Blast

Air velocities of static conditions up to 700 fpm at 0 F and —20 F were employed. (Previous work by Dr. MacArthur revealed that air velocities in excess of these rates were of no advantage in cylindrical pint containers.) The product was placed in an air tunnel in a freezer room at the required temperature. Considerable difficulty was encountered in obtaining uniform air flow in the tunnel. This was particularly true at low velocities of 100 fpm and less. Throughout the experiments the maximum deviation in air flow was about 15 per cent.

In Figure 2 the freezing effect of different air velocities at 0 F is shown. It will be seen, by comparing the freezing points determined by observation with those determined by freezing curve formation, that there is considerable deviation between the two methods. It will be noted, however, that:

1. In all instances the actual freezing point (complete freeze of product) is above the corresponding point determined by the temperature curve.

2. The point determined by the freezing curve is more consistent with the laws of heat transfer.

3. Considering the probable error in determining the freezing point by observation, it can be visualized that with more accurate determinations the two curves in Figure 2 would be approximately parallel.

From a practical standpoint it would appear that even a slow air flow rate markedly speeds up freezing rates. The freezing rate appears to increase up to an air flow rate of about 200-300 fpm. Beyond this rate the increased rate of freezing is not as great, with corresponding increase in air flow.

The point that should be kept in mind is that by using a good air flow at 0 F, the freezing time in pint cartons can be reduced by approximately 45 per cent

Once having established the effect of air flow on freezing rates the next step is to consider the effect of temperature. In Figure 3 are shown freezing rates at —20 F. It will be noticed that the freezing rate under static conditions has been reduced by about 34 per cent of zero static conditions. At 50 fpm air flow the reduction was 48 per cent and at 450-500 fpm the reduction was also 48 per cent when compared with the same air flow at 0 F. Based on a freezing point of 26 F the theoretical reduction of temperature difference should be about 44 per cent.

The probable explanation for the unexpectedly low decrease in freezing rates under static conditions might be explained by the fact that the so-called static conditions were not controlled. In other words there may have been an imperceptibly greater air flow in the air film around the package at 0 F than at —20 F.

When considering effect of air flow and temperature on freezing rates it might be interesting to note the basic reduction of freezing time by minimal practical air flow rate at both 0 F and —20 F. This was found to be about 200-300 fpm at 0 F and possibly 100-200 fpm at —20 F. On the basis of 0 F static being 100 per cent, the reduction in freezing time at 0 F with

300 fpm would be 35 per cent, whereas —20 F with 200 fpm would be 65 per cent.

The point of practical significance is that under zero conditions as great a reduction in freezing rates can be obtained by employing air flow as can be obtained by using a —20 F temperature. Obtaining —20 F temperatures is costly because of the necessity of
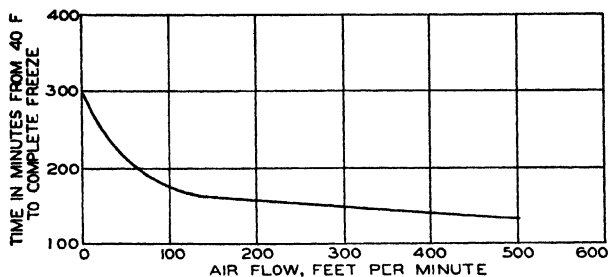

Fig. 3. Freezing rate of strawberry dry pack at —20 F in a freezing tunnel employing various wind velocities (based on temperature trend).

using low suction pressures in the refrigeration system resulting in lower compressor efficiencies, etc. This can be compensated for, of course, by installing more evaporator surface. Either scheme usually involves more expense than the installation of air moving equipment.

The next item considered was the effect of packing on freezing rates. That is, a syrup pack as related to a dry (no sugar) pack. In these experiments pint cartons containing 12.5 ounces net and the syrup pack weighed (12.5 ounces berries plus 9.4 ounces 45 F Brix syrup) 21.9 ounces.

Table 1. Freezing Time in Minutes for Strawberries Packed Dry as Compared to Syrup Pack

Air Velocity	Temp. F	Minutes from 40 F to Complete freeze	
		Dry Pack	Wet Pack
Static	0	455	335
	—20	300	225
	0	365	285
	—20	190	155
	0	270	200
	—20	140	115

It will be noted in Table 1 that in every instance the syrup pack froze in less time than did the dry pack. At 0 F the decrease in time is about 22 to 26 per cent and at —20 F the reduction is 18 per cent at static and 500 fpm air flow.

The reduction in freezing time in the syrup packs is unusual viewed in the light of total heat extraction On the basis of water content the dry pack represents an extraction of about 110 Btu whereas the syrup pack represents about 190 Btu per package. The logical explanation, however, is that the syrup provides greater conductivity of heat from the interior of the package. What is more, this increase of conductivity more than compensates for the heat load added by virtue of its presence.

In the packages where no syrup was used (dry pack) the insulating effect of the air contained in

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the interstices cannot be considered as completely limiting under static conditions.

It is only natural, therefore, that possibly greater advantage in rate of cooling can be gained in high velocity air at low temperatures with liquid packs than with dry packs. Unfortunately, the records are not sufficiently complete in detail to prove this point.

Freezing Rates

The freezing rates on various products were studied. These included asparagus, beans, blueberries, peaches, raspberries, and strawberries. Comparable data are only available on asparagus, peaches, raspberries, and strawberries. The freezing times for these products, wet pack (vegetables in brine and fruits in syrup), static air flow, at 0 F were as follows:

Asparagus	610 minutes
Peaches	450 "
Raspberries	630 "
Strawberries	335 "

The freezing time as indicated above may be slightly longer than actual because this figure is not consistent with other data found in the detailed notes. What is important is that the type of product has a profound influence on rate of freezing. Because of greater variation in commercial conditions than existed in our experimental chambers, magnification of these variations can be expected in practice.

From the commercial standpoint it can be readily seen that much can be done toward speeding up freezing by the judicious use of air movement. A means of measuring this increase can be established as far as fruits and vegetables are concerned.

In many instances, however, the slowest rates of freezing in the experiments may be considered moderately fast in many commercial plants. In any event Dr. MacArthur of the Horticultural Division submitted to the taste panels material frozen similarly to those in the experiment. The results indicated that within the range of the experiment no practical advantages were gained with the fastest freezing rates. Where firmness was achieved, it was frequently offset by loss of color or flavor.

It would appear, therefore, that the chief object in speeding up freezing rates in the commercial fruit and vegetable industry is to avoid "bottlenecks" in the production line.

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CANADIAN WILTSHIRE BACON

XXVIII. CHLORIDE SHIFT IN CURED PORK[1]

By G. A. Grant[2] and N. E. Gibbons[3]

Abstract

Carefully conducted flavor tests and chemical analyses for chloride ion showed that bacon of the Wiltshire type increased in saltiness and water extractable chloride during storage. Enzyme digests of freshly cured pork gave higher chloride recoveries than those obtained in water extraction procedures. On the other hand, recovery of sodium ion in freshly cured material was identical by water extraction, enzyme digestion, or a wet ashing procedure. These results show that the chloride ion is bound by the muscle constituents and that it can be released by enzyme action. This mechanism would explain the increase in saltiness observed in stored bacon.

Introduction

The most serious complaint against Canadian bacon exported to England is its excessive saltiness. This has been supported by flavor tests conducted in England (7). Although it is generally recognized that curing practice is largely responsible for the saltiness of the product, commercial operators have claimed that bacon of the Wiltshire type increases in saltiness during storage. If this is true, then the saltiness of Canadian bacon increases before it is consumed in England. Hence it was thought advisable to investigate the effect of aging on saltiness and to study some of the changes taking place in the muscle tissue.

Experimental and Results

The material in the first experiment consisted of rib-in export backs from 20 hogs. The backs were cut in half and two half-backs allotted at random to each experimental condition. The half-backs were cured to contain 0.2% nitrate at sodium chloride levels of 2, 3, 4, 5, and 6%, and 0.1% nitrate at sodium chloride levels of 2, 4, and 6%. For the material to contain 0.2% nitrate, the pump pickle contained 30% sodium chloride, 1.5% nitrate, and 0.05% nitrite, while the cover pickle contained 28% sodium chloride, 1.5% nitrate, and 0.05% nitrite. Material to contain 0.1% nitrate was cured in similar curing pickles except that the nitrate concentration was reduced to

0.5%. Different sodium chloride levels were obtained by varying the amount of pickle pumped into each half-back and by the time in cure. The material was cured in small tanks for two to four days at 4.4° C. (40° F.) and at a pickle to meat ratio of 20 gal. per 100 lb.

After removal from cure, the pieces were wrapped in waxed paper, over-wrapped in brown kraft, and stored at −1.1° C. (30° F.). Samples were removed from storage at 0, 6, 12, 18, and 24 days and prepared for chemical and biological determinations. Half-backs were sampled as follows: bones were removed and the back cut into slices ¼ in. thick. The slices were then randomized into three lots for chemical analyses, taste panel assessments, and vitamin analyses. Chemical analyses were also done on the cooked samples. The work on the vitamin analyses will be reported in a separate paper (1). The material for chemical and vitamin analyses was trimmed free of most of the fat, minced three times in a food chopper, frozen at −40° C., and stored at that temperature until required for analysis. After the material was thawed, chloride (6), nitrate (4), and moisture contents (6) were deter-mined. Flavor was determined as follows: small pieces of the meat were trimmed free of fat and grilled at 260° C. (500° F.) on a wire screen for six minutes (three minutes on each side). Saltiness was assessed on the following scale: −1 to −5, deficiency; 0, ideal; +1 to +5, excess saltiness. The scoring panel of 15 people was required to score no more than four samples in any morning or afternoon.

Chloride and nitrate contents of the lean meat for each experimental cure, averaged over all storage times, are given in Table I. It is evident that a range of salt concentrations from 3.8 to 5.7% and two levels of nitrate, 0.1% and 0.2%, were obtained in curing the material.

TABLE I

MEAN CHLORIDE AND NITRATE CONTENTS OF WILTSHIRE BACON FROM SEVERAL
CURES, AFTER STORAGE AT −1 1° C (30° F.) FOR 24 DAYS

(Values averaged over all storage times)

Desired nitrate level, %	Desired chloride level, %	Chloride,[a] %		Nitrate,[a] %	
		Raw	Cooked	Raw	Cooked
0.2	2	3.86	6 29	0 16	0 27
	3	4.74	7.05	0 20	0 30
	4	5.01	7.60	0 24	0 37
	5	5 27	7.70	0 21	0 34
	6	5.60	8.13	0.25	0.38
0.1	2	4.35	6 52	0 06	0 09
	4	5.07	7.73	0 06	0.10
	6	5.70	8.53	0.07	0.11
Necessary difference[b]		0.58	0.74	0.027	0.027

[a] *Calculated as the sodium salt.*
[b] *Necessary difference to exceed the 5% level of statistical significance.*

As it is difficult to cure any two pieces of meat to contain exactly the same salt content, the difference between duplicate samples may be large. Hence statistical analyses of the data were employed to establish the significance of any trends obtained in storage. Table II shows the results of an analysis of

TABLE II

ANALYSIS OF VARIANCE FOR THE EFFECTS OF STORAGE ON THE CHLORIDE, NITRATE, AND MOISTURE CONTENTS AND THE FLAVOR OF WILTSHIRE BACON STORED FOR 24 DAYS AT −1.1° C. (30° F.)

Source of variance	Degrees of freedom	Mean square						Flavor
		Chloride		Nitrate		Moisture		
		Raw	Cooked	Raw	Cooked	Raw	Cooked	
Cures	7	3.8**	5 9**	0 066**	0.155**	0.7	16.0	1.50**
Time	4	2.1**	5.2**	0.008**	0.039**	2.6	1.7	2.20**
Cures × time	28	0.4	0.6	0.001	0.003	0.8	10.4	0.14
Between duplicates	40	0.4	0.7	0.002	0.002	1.4	25.9	0.27

** Indicates 1% level of statistical significance.

variance: the time of storage had significant effects on the chloride and nitrate contents and on flavor, the moisture content was unaffected, and all the salt levels behaved similarly. The magnitude and direction of the significant effects are given in Table III. Flavor scores showed a significant

TABLE III

EFFECTS OF TIME OF STORAGE ON THE MEAN CHLORIDE AND NITRATE CONTENTS AND FLAVOR SCORE OF WILTSHIRE BACON STORED AT −1.1° C. (30° F.) FOR 24 DAYS

(Values averaged over all cures)

Time, days	Chloride,[a] %		Nitrate,[a] %		Flavor score[b]
	Raw	Cooked	Raw	Cooked	
0	4.44	6.68	0.13	0.17	0.7
6	4.73	7.04	0 14	0.26	0.9
12	5.09	7.66	0.17	0.23	1.3
18	5.15	7.82	0.16	0.27	1.6
24	5.35	8.03	0.18	0.30	1.6
Necessary difference[c]	0.46	0.56	0.027	0.024	0.4

[a] Calculated as the sodium salt.
[b] Saltiness ratings only.
[c] Necessary difference to exceed the 1% level of statistical significance.

increase in saltiness during storage, first evident after 12 days. This coincided with significant increases in chloride and nitrate contents of both the cooked and uncooked meat. From these results the increase in saltiness on storage appears to be real and substantial.

Comparison of Analytical Methods

It was necessary to consider the analytical methods involved and to check the results of the previous experiment by employing another analytical procedure. The investigations of White (6) on cured meat showed that ashing the meat by the A.O.A.C. method and titrating by the Volhard procedure (3) produced lower chloride values than extracting the meat with hot water and titrating by the Mohr procedure (6). There are two possible explanations for these results; underestimation of chloride by loss of chlorine during ignition of the meat in the ashing procedure or the overestimation of chloride ion by titration of materials other than chloride in the water extract of the meat. The latter was checked by adding known amounts of pure sodium chloride to water extracts of fresh pork. Complete recoveries of the chloride ion were obtained. Therefore it was assumed that, owing to loss of chlorine during ignition, the ashing procedure underestimates chloride content and that water extraction and direct titration is the better procedure.

The increase in chloride content of stored bacon was rechecked by the ashing and water extraction procedures. The results are given in Table IV.

TABLE IV

THE ESTIMATION OF SODIUM CHLORIDE IN WILTSHIRE BACON STORED FOR 0 AND 24 DAYS AT −1.1° C. (30° F.) BY DRY ASHING AND WATER EXTRACTION PROCEDURES

(Means of duplicate determinations)

Sample No.	Sodium chloride, %			
	Dry ashing		Water extraction	
	0 days	24 days	0 days	24 days
1	2.1	3.3	2.4	3.5
2	3.5	4.7	3.6	5.1
3	4.8	5.8	4.9	6.2

The chloride content as determined by the ashing procedure was lower than that determined by water extraction. This is in agreement with the previous results reported by White (6). Although the chloride values obtained by the two methods were different the increase in chloride content on storage of bacon was evident by both procedures. Therefore the increase in saltiness of bacon on storage does not depend on the analytical procedure used to estimate the chloride ion.

Diffusion of Chloride or Moisture from Fat to Lean

As only the lean meat had been analyzed it was possible that chloride and nitrate had diffused from the fat to the lean. This was investigated but since nitrate is unstable owing to bacterial action, only changes in the chloride were followed.

This experiment employed six export backs divided into 30 pieces. The pieces were cured to give sodium chloride contents of 2, 4, and 6%, then stored and sampled as in the previous experiment. Chloride and moisture contents were determined on the fat and lean portions of each piece. Moisture content in the fat and lean and chloride content in the lean were determined as previously described (6). Chloride content in the fat was determined according to the method of the A.O.A.C. (3) in which the sample is ashed, leached with hot water, and the chloride of the extract determined by the Mohr procedure.

Chloride and moisture contents of the fat and lean meat for each cure, averaged over all storage times, are given in Table IV. It is evident that only small amounts of chloride and moisture diffuse into fat during curing. Statistical analysis of the data shows that the time of storage had a significant effect on the chloride content of both lean and fat (Table V). As pork fat

TABLE V

CHLORIDE AND MOISTURE CONTENTS OF THE LEAN AND FAT OF WILTSHIRE
BACON STORED AT −1.1° C. (30° F.) FOR 24 DAYS
(Mean values averaged over all other conditions)

Desired chloride levels, %	Lean meat		Fat	
	Chloride, %	Moisture, %	Chloride, %	Moisture, %
2	2.82	71.6	0.37	7.68
4	4.44	71.2	0.59	7.52
6	6.10	70.9	0.63	7.33
Necessary difference[a]	0.56	0.86	0.14	1.02

[a] *Necessary difference to exceed the 1% level of statistical significance.*

contains appreciable amounts of connective tissue, it is not surprising that it exhibits somewhat the same behavior as lean meat. It is evident that the increase in water extractable chloride could not be attributed to diffusion of the chloride or moisture from the fat to the lean.

Release of Bound Chloride by Enzymes

The increase in water extractable chloride on storage may be explained by fixation of a portion of the chloride by the freshly cured muscle and subsequent release of chloride through enzyme action, or otherwise. As it was possible that fixation of either the sodium or chloride ion, or both, may have occurred, both sodium and chloride were determined on freshly cured pork in the next experiment.

Freshly cured backs were trimmed free of fat and bone, minced, and samples subjected to water extraction, enzyme digestion, and wet ashing procedures. Water extraction was carried out by a previously described method (6). In the enzyme digest procedure, 10 gm. of meat was digested for 18 hr. at 37° C. with 0.2 gm. of papain and 0.2 gm. of takadiastase in 100 ml. of solution adjusted to pH 4.6. Wet ashing was done with concentrated sulphuric and nitric acids. Chloride was determined by the Mohr procedure and sodium by precipitation as sodium zinc uranyl acetate (5, p. 878). From Table VI, it is

TABLE VI

EFFECTS OF STORAGE ON THE CHLORIDE AND MOISTURE CONTENTS OF THE FAT AND LEAN
PORTIONS OF WILTSHIRE BACON STORED AT −1.1° C. (30° F.) FOR 24 DAYS

(Mean values averaged over all other conditions)

Time, days	Lean meat		Fat	
	Chloride,[a] %	Moisture, %	Chloride,[a] %	Moisture, %
0	3.64	72.3	0.37	7.31
6	4.21	71.5	0.41	7.64
12	4.85	70.6	0.48	7.08
18	4.62	71.0	0.67	8.43
24	4.94	70.7	0.68	7.05
Necessary difference[b]	0.72	1.11	0.14	0.39

[a] Calculated as the sodium salt.
[b] Necessary difference to exceed the 1% level of statistical significance.

evident that the recovery of the sodium was similar by water extraction, enzyme digestion, or wet ashing procedures, but that digestion with papain and takadiastase gave higher recoveries of chloride than the water extraction procedure. These results show that the chloride is bound by the muscle tissues, that it can be released by enzyme action, and that the sodium ion is not bound.

TABLE VII

CHLORIDE AND SODIUM CONTENTS OF FRESHLY CURED WILTSHIRE
BACON BY DIFFERENT METHODS OF ANALYSIS

Sample No.	Sodium, %			Chloride, %		Chloride, %
	Water extract	Enzyme digest	Wet ash	Water extract	Enzyme digest	Difference between enzyme digest and water extract
1	0.73	0.80	0.81	1.22	1.52	0.30
2	0.94	0.95	0.93	1.48	1.96	0.48
3	2.05	2.06	2.06	3.19	3.63	0.44

Discussion

The increase in saltiness in cured pork may be satisfactorily explained as follows: while pork is curing in brine solution, some of the chloride ions are bound by the muscle tissues while the sodium ions remain in the outer tissue fluid with the excess sodium chloride.   When the cured pork is stored, bacterial or autolytic enzymes or both break down the muscle tissue and the chloride is released.   The freed chloride then reunites with the sodium to form sodium chloride.   If it is assumed that the bound chloride is not released on mastication, this mechanism satisfactorily explains the increase in saltiness that occurs in stored bacon.   An increase in nitrate also occurred during storage, and it is possible that the nitrate ion is behaving similarly to the chloride ion.   This would further enhance the saltiness during storage, as it has been shown that sodium nitrate imparts a salty flavor to cured products (2).   As the concentrations of potassium and other ions were not determined, no calculations have been made to determine the amount of chloride bound by the muscle tissue or accounted for as sodium chloride.   More work is required to ascertain the concentration of chloride that is bound by the muscle tissue, and the factors governing the reaction.

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CANADIAN WILTSHIRE BACON

XXIX. CHANGES IN THE THIAMINE, RIBOFLAVIN, AND NIACIN CONTENTS PRODUCED BY CURING, STORAGE, AND COOKING

By Paul R. Gorham

CANADIAN WILTSHIRE BACON

XXIX. CHANGES IN THE THIAMINE, RIBOFLAVIN, AND NIACIN CONTENTS PRODUCED BY CURING, STORAGE, AND COOKING[1]

By Paul R. Gorham[2]

Abstract

Significant amounts of thiamine and niacin, but not of riboflavin, were lost during the process of curing pork half-backs in brine pickle on a semicommercial scale. This loss was not related to the final chloride or nitrate content of the meat. Cured half-backs stored at 30° F. ($-1.1°$ C.) for 24 days lost significant amounts of thiamine and riboflavin, but the niacin content remained unchanged. Curing caused a significant increase in the percentage of niacin retained after cooking. During the storage of cured half-backs, the percentage of thiamine retained after cooking increased significantly.

Introduction

As part of an extensive series of investigations of Canadian Wiltshire bacon aimed at improving the quality of the product being shipped overseas, a study was undertaken of the losses of three of the more important B-vitamins arising from the use of different curing pickles, storage, and cooking.

Jackson *et al.* (5) found that with the "wet cure" method of processing bacon the loss of thiamine and niacin exceeded that of riboflavin, while in "dry cured" bacon the greatest loss occurred with riboflavin. Little or no information was available on changes in the B-vitamin content of bacon caused by storage or cooking.

Materials and Methods

In the preceding paper (2) full details have been given of the curing, storage, cooking, and sampling of the 80 half-backs of pork from which samples were obtained for vitamin analyses.   These half-backs were cured to produce final chloride contents ranging from 2 to 6% at nitrate levels of 0.1% and 0.2%. Duplicate half-backs from each of the eight cures were withdrawn from storage at 30° F. every six days.   Fat and fiber were trimmed away and the lean meat uniformly sliced.   The slices from each half-back were distributed at random into four equal lots, of which two were available for vitamin analyses.   Of these, one lot was thoroughly ground and mixed by two passages through a meat grinder, while the slices in another lot were cooked by broiling in a domestic electric oven for three minutes on each side and then ground. The ground samples were stored at $-40°$ F. pending analysis.

Four uncured half-backs were treated in a comparable manner to provide samples of fresh and cooked pork for analysis.

Samples were withdrawn from −40° F. and allowed to thaw for 24 hr. at 40° F. A 15 gm. portion of uncooked or a 10 gm. portion of cooked material was extracted with papain and takadiastase and made up to a volume of 200 ml. (7). A dilution of 25 ml. per 100 ml. was used for the assay of thiamine by a modification (11) of the thiochrome method (4, 10). Riboflavin was assayed fluorometrically, using the antimony trichloride–ethanol procedure (7). A dilution of 2.5 ml. per 100 ml. was used for the microbiological assay of niacin by the method of Krehl, Strong, and Elvehjem (6).

Moisture determinations, made on the ground samples by vacuum-drying at 100° C. (2), were used to calculate the results on a moisture-free basis. Changes caused by cooking were expressed as the percentage vitamin retained.

Results

Fresh pork was found to contain, on the average, 66 μgm. of thiamine, 6μgm. of riboflavin, and 220 μgm. of niacin per gram dry weight. As was expected from work reported by others (1, 13), different carcasses contained different amounts of the three vitamins; the percentage retained after cooking also showed a considerable degree of variability (Table I).

TABLE I

VITAMIN CONTENT OF FRESH PORK AND PERCENTAGE RETAINED AFTER COOKING

(Values represent means of duplicate determinations)

Half-back	Uncooked			Cooked		
	μgm./gm. dry wt.			Percentage retained		
	Thiamine	Riboflavin	Niacin	Thiamine	Riboflavin	Niacin
1	68.3	5 55	213 3	59.0	76.6	69.9
2	79.0	6.84	258.6	65.0	77.2	71 4
3	52.8	6.48	229.5	75.8	75.5	66 3
4	64.4	5.04	177.7	77.7	86.7	68 8
Mean	66.1	5.98	219.8	69 4	79.0	69.1

Analyses of variance were used to determine whether significant losses of vitamins occurred during the curing process, and whether the percentage retained after cooking was affected. The results are presented in Table II. Curing caused significant losses of thiamine and niacin but caused little change

in riboflavin content. The percentage of all three vitamins retained after cooking was somewhat higher in cured pork than in uncured pork, but the difference was significant for niacin only.

TABLE II

THE EFFECT OF THE CURING PROCESS UPON THE VITAMIN CONTENT OF PORK
AND THE PERCENTAGE RETAINED AFTER COOKING

| Treatment | Uncooked | | | Cooked | | |
| | μgm./gm. dry wt. | | | Percentage retained | | |
	Thiamine	Riboflavin	Niacin	Thiamine	Riboflavin	Niacin
Uncured[a]	66.1	5.98	219.8	69.4	79.0	69.1
Cured[b]	51.9	5.82	152.0	77.0	86.2	80.9
Necessary difference, 5% level	12.6	—	54.3	—	—	9.3

[a] *Values represent means of four determinations in duplicate.*
[b] *Values represent means of 16 determinations.*

The vitamin contents of samples subjected to various cures did not differ significantly. However, there were significant differences in the percentage of riboflavin and niacin retained after cooking, the highest retentions being associated with low chloride content (Table III).

TABLE III

THE EFFECT OF DIFFERENT CURES UPON THE PERCENTAGE OF THREE VITAMINS RETAINED
AFTER COOKING

(Each value represents the mean of 10 determinations)

| Vitamin | Nitrate, 0.2% | | | | | Nitrate, 0.1% | | | Necessary difference, 5% level |
| | Chloride, % | | | | | Chloride, % | | | |
	2	3	4	5	6	2	4	6	
Thiamine	83.7	83.3	78.8	72.1	84.7	82.8	85.1	77.8	—
Riboflavin	94.0	90.2	87.7	80.5	86.9	86.8	92.5	85.5	6.5
Niacin	87.6	89.6	84.7	79.6	81.3	91.0	85.2	84.0	7.5

There was a significant loss of thiamine and riboflavin, but not of niacin, from cured pork stored at 30° F. for 24 days (Table IV). During this period the percentage of thiamine retained after cooking gradually increased until the difference attained statistical significance. No such increase occurred with riboflavin or niacin.

TABLE IV

THE EFFECT OF STORAGE AT 30° F. UPON THE VITAMIN CONTENT OF CURED PORK AND THE
PERCENTAGE RETAINED AFTER COOKING

(Each value represents the mean of 16 determinations)

| Vitamin | Storage time, days | | | | | Necessary difference, 5% level |
	0	6	12	18	24	
	μgm./gm. dry wt.					
Thiamine	51.9	38 9	46.0	41 4	37 8	9 5
Riboflavin	5.82	5.31	5.39	5 64	4.78	0 58
Niacin	152.0	162.4	151.5	164 9	150 4	—
	Percentage retained after cooking					
Thiamine	77.0	78.3	75.3	88 6	85 8	7 5
Riboflavin	86 2	87 8	87.6	87.7	90 8	—
Niacin	80 9	86.2	83.1	88.8	87.7	—

Discussion and Conclusions

The loss of significant amounts of thiamine and niacin during the curing process (Table II) suggests that these two vitamins leach into the pickle, whereas riboflavin does not. After pork hams have been wet-cured and smoked, they have been found to lose more thiamine than niacin or riboflavin (16, 17). It was thought possible that differences in vitamin content between the present materials, subjected to eight different cures, might have been obscured, in part, by occasional failure to attain the desired chloride levels. However, even when the actual chloride contents are considered there is still no well-defined relation between vitamin content and the chloride or nitrate content of the cured pork.

The average vitamin retentions during the storage of pork products, reported by various investigators, are shown in Table V. Rice, Fried, and Hess (14) found that ground flank muscle spoiled after storage for 14 days at 40° F. They attributed subsequent high vitamin retentions to synthesis by spoilage microorganisms. Under approximately comparable conditions of storage, cured half-backs lost somewhat more thiamine than fresh hams, fresh loins, or fresh or cured ground flank muscle; all lost about the same amount of riboflavin; but, unlike fresh hams, fresh loins, or cured ground flank muscle, cured half-backs lost no niacin.

Losses of thiamine, niacin, and riboflavin occur during the cooking of fresh pork (1, 8, 9, 12, 14, 15, 16). These losses increase the more the pork is cooked, with loss of thiamine greatly exceeding losses of the other two vitamins (5). Under the cooking conditions used in these experiments, both in uncured and cured pork, the percentages of thiamine and niacin retained were about

TABLE V

RETENTIONS OF THIAMINE, RIBOFLAVIN, AND NIACIN IN STORED PORK PRODUCTS REPORTED
BY VARIOUS INVESTIGATORS

Source of data	Material	Storage conditions		Average retention, %		
		Temp., °F.	Time, days	Thia-mine	Ribo-flavin	Niacin
Schweigert, McIntire, and Elvehjem (16)	Fresh hams	24.8	14	92	85	92
Rice, Fried, and Hess (14)	Fresh loins	40	6	101	93	81
			10	107	100	63
			20	95	90	64
			68	· 97	94	78
	Ground flank muscle	40	7	95	100	102
			14	93	104	97
			21	95	118	97
			28	87	154	101
	Ground flank muscle, nitrate–nitrite–glucose cured	40	14	95	105	90
			56	91	120	93
Gorham (calculated from Table IV)	Half-backs, sodium chloride–nitrate–nitrite cured	30	6	75	91	106
			12	89	93	100
			18	80	97	108
			24	73	82	99

equal and lower than the percentage of riboflavin retained (Table II). The higher percentage of niacin retained after cooking in cured than in uncured samples may bear some relation to the type of cure since it decreased as the sodium chloride content increased (Table III). Initially, the percentage of thiamine retained after cooking was not significantly affected by curing (Table II), as Greenwood and associates have reported (3), but, during subsequent storage, the thiamine retained after cooking increased to a significant degree (Table IV). There is need for further study of curing as it affects the amount of vitamin retained after cooking. The variability observed in the present study may be reduced by the use of a more rigidly standardized cooking procedure.

These experiments, conducted on a semicommercial scale, indicate that an appreciable loss of thiamine, riboflavin, and niacin occurs during the curing of pork in fresh brine pickle and its subsequent storage at 30° F. In commercial practice, however, where many whole backs are cured in brine that is used repeatedly, the losses may not be as great.

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THE REDUCING SUGAR CONTENT OF FROZEN EGG AS AN INDEX OF THE BACTERIAL CONTENT

By C. K. Johns

THE REDUCING SUGAR CONTENT OF FROZEN EGG AS AN INDEX OF THE BACTERIAL CONTENT[1]

By C. K. Johns[2]

Abstract

Reducing sugar determinations have been proposed as an over-all measure of both bacteriological and chemical changes in frozen egg. When applied to commercial products, the technique recommended has been found to yield poorly reproducible results, while reducing sugar readings show no correlation with viable or microscopic counts of bacteria. Few of the bacteria isolated from commercial melange were able to ferment glucose.

In assessing the quality of frozen egg, the bacterial content deserves consideration, since a high count usually denotes either low grade breaking stock or faulty plant operations. Furthermore, a high count product may spoil during defrosting, while conditions that allow considerable bacterial growth may permit the multiplication of pathogenic types, with a consequent possible public health hazard (2, 3, 4, 7, 10, 11, 12).

However, quality in frozen egg includes other aspects in addition to the bacterial. The type of breaking stock, or the growth of bacteria in the melange, may be reflected in chemical changes, some of which give rise to undesirable flavors and odors in the product. Consequently, various chemical tests have been suggested (6). Among these is the determination of the reducing sugar content. This test has recently been advocated as an over-all indication of egg quality by Pearce and Reid (8), who report that reducing sugar values decreased with increasing numbers of bacteria. Such a test, giving promise of indicating both chemical and bacteriological aspects of egg quality, would be extremely useful in the regulatory control of frozen egg.

It is generally recognized, however, that the bacterial content of a food product has to be well up in the millions per gram before the effect of bacterial activity can be measured by ordinary chemical tests. Consequently, there was reason to doubt the effectiveness of the reducing sugar test as a means of measuring or reflecting the bacterial content. With this in mind, studies were undertaken to determine the degree of correlation between bacterial content and reducing sugar content of commercial frozen egg, and also between each of these measures and plant conditions and operations as observed at the time of sampling. However, since only a few plants were operating when the frozen material was sampled, such observations were obviously of limited value.

Materials and Methods

During October and November, 1946, Mr. D. A. Fletcher of the Poultry Products Marketing Service, Department of Agriculture, visited a number of plants between Ottawa and the Pacific coast where eggs were broken and frozen for the domestic trade.   At each plant, cores of whole egg, white, and yolk were bored, using a sterile 1 in. corer and an electric drill.   One portion of the core was placed in a sterile stoppered 7/8 in. X 6 in. Pyrex test tube and kept frozen.   When all samples from any city had been collected, they were carefully packed in dry ice and shipped by air express to Ottawa.   Here they were rapidly thawed in a water bath and analyzed for (a) total viable count on standard milk agar at 32° C. for three days, (b) direct microscopic count, and (c) coliform count on Bacto violet red bile agar at 37° C. (1). A second portion of each sample was analyzed by Mr. Fletcher in the field for reducing sugars and total solids, using for the former the method employed by Pearce and Reid (8).   An additional determination of reducing sugar was made by the Division of Chemistry, Science Service, on the portion of sample remaining after bacteriological analysis, in order to establish reproducibility of results between two workers using the same reagents, etc.

Results

Fig. 1 shows data obtained from 59 samples of frozen whole egg.   The lack of correlation is obvious.   The majority of samples with viable counts below 1,000,000 per ml. were below the minimum of 300 mgm. %* reducing sugar that had been suggested for Grade A whole egg, while a number of those with very high counts were well above this limit.
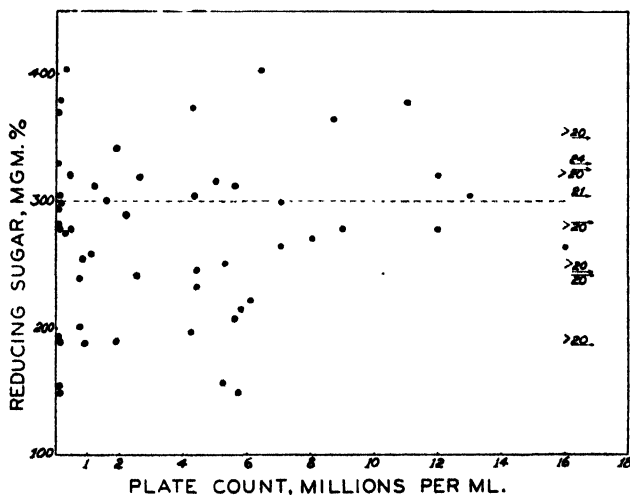

FIG. 1.   *Reducing sugar content vs. plate count of 59 samples of commercial frozen whole egg.*

This represents the number of milligrams in 100 gm., or the percentage X1000.

Fig. 2 shows similar data obtained from frozen yolk and white. Again, the lack of correlation is obvious.
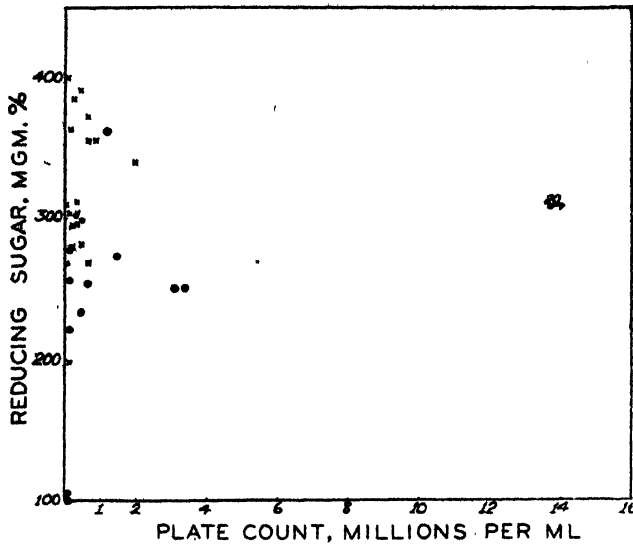

FIG. 2. *Reducing sugar content vs. plate count of commercial frozen yolk (14 samples) and white (20 samples).* ● = *yolk;* × = *white.*

In Figs. 1 and 2 the reducing sugar values are those determined by Mr. Fletcher. The poor agreement between these values and those obtained by the Division of Chemistry is evident from the data in Fig. 3. In general,
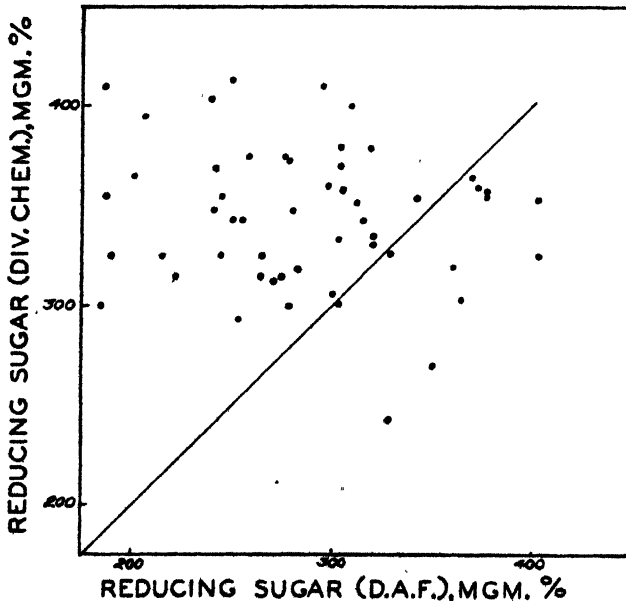

FIG. 3. *Reducing sugar contents of 53 samples of commercial whole egg as determined by two different workers.*

each of the two analysts obtained reasonably satisfactory agreement between their own duplicate determinations.   However, subsequent studies, in which the same two workers made parallel determinations in the same laboratory, failed to yield results in close enough agreement to warrant the use of this test in its present form.

The results of an attempt to relate plant conditions and practices to reducing sugar and bacterial contents appear in Table I.   While the reducing sugar

TABLE I

RELATION BETWEEN QUALITY OF BREAKING STOCK, PLANT CONDITIONS AND PRACTICES, ETC., AND BACTERIOLOGICAL AND CHEMICAL DATA

Sample No.	Reducing sugars, mgm. %		Viable count per ml.	Microscopic count per ml.	Coliform count per ml.
	D.A.F.	Div. Chem.			

A. Eggs broken and frozen under ideal conditions

31	370	364	75,000	—	<100
32	342	353	1,900,000	5,600,000	<100
93	403	330	6,400,000	3,700,000	51,000
94	377	355	11,000,000	3,200,000	30,000

B. Melange produced under sanitary conditions but lacking facilities for fast freezing

86	222	315	6,000,000	7,900,000	400
87	215	325	5,800,000	9,200,000	<100
88	258	375	1,100,000	3,200,000	78,000
89	239	403	760,000	1,800,000	13,000
90	207	395	5,600,000	6,300,000	38,000
91	193	465	60,000	—	14,000
92	190	325	1,900,000	5,300,000	56,000

C. Melange from candling bench mixed eggs

1. Placed in good freezer fairly promptly

70	364	302	8,700,000	12,000,000	400
71	403	354	300,000	3,300,000	<100
74	350	271	>20,000,000	33,000,000	<100
75	298	360	7,000,000	20,000,000	200

2. Placed in poor freezer at end of each day's operations

54	244	325	>20,000,000	>33,000,000	>100,000
55	242	368	20,000,000	33,000,000	10,000
62	250	—	3,100,000	5,100,000	100
63	273	378	1,500,000	1,900,000	<100

values as determined by Mr. Fletcher appear to correlate well with plant practices and conditions, an inverse relationship exists when the Division of Chemistry values are used.   Bacterial counts appear to show little or no

correlation with plant practices. However, it should be pointed out that in most plants such important points as adequacy of sanitizing of utensils and equipment could not be checked, since the sampling was conducted many months after breaking operations had ended.

Discussion

The lack of correlation between bacterial numbers and reducing sugar content found in these studies is not altogether surprising. Even if a close correlation existed between the reducing sugar content and the bacterial content of the egg at the moment of breaking, this might easily be upset by a heavy pick-up of bacteria from equipment. If no opportunity existed for subsequent bacterial growth in the melange before freezing, this would not affect the reducing sugar content. Furthermore, various workers have shown that a high proportion of the bacteria present within the egg are unable to utilize reducing sugars in their growth processes. Finally, there is little published information concerning the effect of climate, season, storage, and other factors upon the reducing sugar content, and little is known as to how widely normal eggs vary in this constituent.

The lack of agreement between our results and those reported by Pearce and Reid (8) concerning the correlation between bacterial numbers and reducing sugar content may arise from the fact that they worked with Grade A eggs, broken out under nearly aseptic conditions in the laboratory, while our samples were obtained from a number of commercial plants breaking and freezing lower grades of eggs. It is probable that sugar-fermenting types, coming from the shells, were more common in their melange; Gillespie (5) has noted that Gram-positive cocci make up a very considerable part of the normal flora of the egg shell, while they occur infrequently in commercial melange. This we had also found to be true in previous studies. For further confirmation, plates from five samples of frozen whole egg in the present studies were selected; from each 50 adjacent colonies were fished onto slants of Difco tryptone glucose extract milk agar (1) containing brom-cresol purple indicator. After incubation at room temperature for two to three days, the slants were examined and any evidence of acid formation recorded. Results were as follows:

Sample No.	Reducing sugar, mgm. %		Viable count per ml.	Acid formers, %
	D.A.F.	Div. Chem.		
42	250	343	5,300,000	< 2
45	373	359	4,300,000	2
52	275	314	280,000	12
54	244	325	>20,000,000	< 2
55	242	368	>20,000,000	< 2

With such small proportions of acid-forming bacteria, a high degree of correlation between reducing sugars and bacterial numbers could scarcely be expected.

Although the data are not shown, direct microscopic counts also showed no better correlation with reducing sugar readings than did viable counts. At first it appeared that the content of coliform organisms might afford a good index to the bacteriological and sanitary quality, but analysis of additional samples revealed many anomalies between coliform content, other bacteriological counts, and reducing sugar content.

In 1921, discussing the proposal to use the reducing sugar content, Redfield (9) remarked: "Whether Todd's general deduction (p. 512), that the dextrose content is a more reliable indication of the age of the eggs than the ammonia nitrogen, is true or not depends entirely upon the bacterial flora of the eggs and whether the predominating changes during aging are fermentative, proteolytic, or lipolytic. Since they may be any of these, the quality of eggs cannot be judged by any one chemical method." Our findings appear to underline the last sentence. While it would be most convenient to have a single chemical test that would indicate over-all quality in frozen egg, in view of the considerations indicated by Redfield, it seems unlikely that one will be found. Apparently, it will be necessary to resort to a combination of bacteriological and chemical examinations, supplementing organoleptic tests, in order to obtain a true picture of the quality of frozen egg products.

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DISCOLORATION

of packaged red meat*

One of the last major food items to resist pre-packaging for retail sale is meat. This product is now beginning to be packaged for self service and in most places where the experiment has been tried it has met with considerable success from the viewpoint of sales (1, 2)[1]. Some technical difficulties must be overcome, however, before packaging is completely successful.

One of the greatest difficulties is that of discoloration. Fresh red meats (e.g., beef) when in contact with packaging material such as cellophane turn a dark, unattractive, brown color in from three to 24 hrs.

Several attempts have been made to prevent or reduce discoloration Pieces of fat may be placed on the surface of the meat to prevent the wrapper from coming into contact with it (1). Steaks, etc., may be placed in fibreboard trays with sides high enough to prevent the cellophane top from touching the meat (1). The pieces of meat may be stacked with sheets of "peach market paper"[2] between the slices for 24 hrs. and then wrapped in cellophane. This treatment delays discoloration considerably (2). A special grade of cellophane (MSADT) which has a moistureproofing lacquer on one side only (uncoated side used next to meat) has been found to reduce discoloration somewhat, but it is not entirely satisfactory when used in conjunction with present-day display equipment If showcase temperatures were lowered to about 32 deg. F., MSADT cellophane might solve the problem.

The constituent of meat responsible for its color is known as myoglobin or muscle hemoglobin. While muscle hemoglobin and blood hemoglobin are probably not identical chemically, they react similarly and, for the purposes of this paper, will be considered together

Lack of oxygen found to be the cause; sodium bisulfite treatment of wrapper reduces problem and development of an oxygen-permeable transparent film may finally solve it.

By C. G. LAVERS†

under the common term of hemoglobin. As indicated by the name, hemoglobin consists of two parts; heme, the iron-containing fraction, and globin, the protein fraction. The forms of hemoglobin in which we are chiefly interested are:

Reduced hemoglobin or, simply, hemoglobin. In reduced hemoglobin the iron is in the ferrous (Fe^{++}) state. The color of this compound is dark red or purple and is responsible for the dark color in the interior of meat which can be seen when it is first cut.

Oxyhemoglobin. In oxyhemoglobin the iron is still in the ferrous (Fe^{++}) state, but this compound contains more oxygen than reduced hemoglobin. The oxygen is held only in loose combination and can be removed with a vacuum pump. No true oxidation has taken place, only oxygenation. Oxyhemoglobin is bright red and is responsible for the attractive bright red color of meat. When meat is cut and a surface exposed, the dark-red hemoglobin takes up oxygen from the air, forming bright-red oxyhemoglobin.
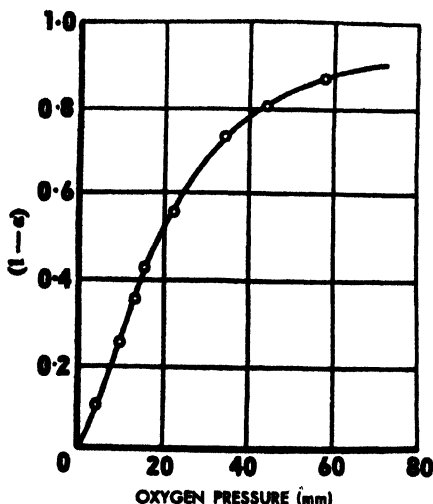
Methemoglobin and other hemoglobins. Methemoglobin contains no more oxygen than reduced hemoglobin, but the iron has been oxidized to the ferric

1. **OXYGEN DISASSOCIATION curve of hemoglobin. 1-α is fraction of hemoglobin in form of oxyhemoglobin.**

(Fe^{+++}) state, a true oxidation. This material is dark brown in color.

In addition, hemoglobin will combine with various substances (carbon monoxide, nitric oxide, hydrogen sulphide) to form stable compounds.

The most common and probably simplest method of identifying hemoglobin compounds is by measuring the wave lengths of light absorbed by them, i.e., their absorption spectra. Each compound has a characteristic absorption spectrum by which it can be identified. Several of these are described in Table I (3).

Experimental

The discoloration of red meat has been reported as a reaction between the meat and the coating on cellophane (5). However, it was of interest, firstly, to see if this discoloration was caused only by a particular wrapping material, such as the moistureproof coating on cellophane, and, secondly, to determine the nature of the brownish pigment.

Samples 2 by 2 in. were cut from the following packaging materials: MSAT cellophane, MSADT cellophane, LSAT cellophane, cellulose nitrate, polyethylene, Saran, Pliofilm, ethyl cellulose and MST cellophane coated with a flexible wax composition. These patches were placed on the surface of a red beefsteak and the meat was stored in a refrigerator at 45 deg. F. Without exception, considerable discoloration had occurred beneath all the patches within 24 to 48 hrs. Discoloration, therefore, was not a function of a particular packaging material.

After 48 hrs., the patches were removed from the meat and a thin slice $1/16$ to $1/8$ in. thick was taken from each of the discolored areas. Each of the samples was trimmed to 2.00 gm. and then placed in a separate flask, 20 ml. of distilled water added and the flask shaken gently for half an hour. The extract was then filtered and its absorption spectrum measured using the Beckman quartz spectrophotometer.

The spectrum of each of the extracts showed absorption maxima at 4,980-5,020, 5,380-5,420, 5,770-5,800 and 6,300-6,340 Angstrom units. This corresponds almost exactly with the regions of light absorption by methemoglobin as shown in Table I. Small variations in the maxima were probably caused by the presence of materials other than methemoglobin, but it was the predominant colored material present. Discoloration, therefore, was caused by the change of oxyhemoglobin to methemoglobin.

Since discoloration is caused by oxidation and since it is well known that sodium bisulfite will preserve the color of meat, wrappings treated with reducing agents were tried as a means of preventing discoloration. Tests using MSADT cellophane treated with ascorbic acid or sodium bisulfite showed that the former was of little value, while the latter was quite effective. MSADT cellophane soaked in a 5% solution of sodium bisulfite for 10 min., rinsed in distilled water and dried, maintained the red color of meat for 72 hrs. at 45 deg. F., both in patch tests and completed packages.

To verify that the bisulfite was acting only as a reducing agent and not forming a red-colored combination product with hemoglobin, a sample of meat was taken from beneath a patch of bisulfite-treated MSADT cellophane and its extract analyzed spectrophotometrically. The analysis showed only oxyhemoglobin. As a further check, a sample of red meat was placed in a vacuum desiccator which was then evacuated until the only color visible in the meat was the dark red of reduced hemoglobin. The desiccator was then filled with nitrogen and a piece of bisulfite-treated MSADT cellophane placed on the surface of the meat. The red color did not return to the meat, showing that bisulfite is incapable of producing a red color in the absence of oxygen. Its only apparent action in air is to prevent a change from oxyhemoglobin to methemoglobin.

This work shows that packaging materials such as MSADT cellophane treated with a suitable reducing agent can be used for wrapping meat without causing discoloration. Tests showed that the water extract of peach market paper was capable of acting as a reducing agent, so it is probably a reducing agent that gives this paper its power to preserve the color of meat.

It is not suggested that bisulfite is the only reducing agent suitable for this purpose and, indeed, it may have some serious drawbacks. However, the work indicates the lines along which future research may proceed.

It has been shown that the discoloration of red meats is due to a change from oxyhemoglobin to methemoglobin. The question may then be asked, "Why should

Compound	No. of bands	Wave length of absorption band, Angstrom units			
Reduced hemoglobin	1	5,650		.	..
Oxyhemoglobin	2	5,769	5,448		
Methemoglobin	4	6,300	5,780	5,400	5,000
Carbon monoxide hemoglobin	2	5,709	5,350	.	
Sulphemoglobin	3	6,180	5,780	5,400	.
Nitric oxide hemoglobin	2	5,745	5,360		

oxidation be speeded up by the application of a packaging material such as cellophane, which cuts off the supply of oxygen from the air?" The explanation for this was found in the mechanism of the oxidation of hemoglobin.

Mechanism of the discoloration reaction

Methemoglobin and reduced hemoglobin form a reversible oxidation-reduction system of the ferri-ferrous type (4). Oxyhemoglobin is not an intermediate step in the formation of methemoglobin. That is, the path of the discoloration reaction is: oxyhemoglobin changes to reduced hemoglobin and the hemoglobin then changes to methemoglobin. To speed up the oxidation of hemoglobin, it is therefore necessary to have conditions favoring the formation of reduced hemoglobin from oxyhemoglobin. Fig. 1 shows the fraction of hemoglobin in the form of reduced hemoglobin under various oxygen pressures at 30 deg C. The percentage of reduced hemoglobin increases rapidly as the oxygen pressure falls below 80 mm. We would therefore expect the discoloration to proceed more rapidly at low oxygen pressure. Fig. 2, giving velocity constants for the oxidation of reduced hemoglobin to methemoglobin, shows that this is so. The maximum reaction rate occurs at about 20 mm. oxygen pressure. At this point the oxygen pressure is sufficiently low to permit the formation of appreciable reduced hemoglobin from oxyhemoglobin (about 50%, Fig. 1) and it is still high enough to carry out the oxidation of reduced hemoglobin to methemoglobin.

When an oxygen-impermeable sheet such as MSAT cellophane is placed on meat, we get conditions suitable for rapid oxidation. The supply of oxygen from the air is cut off, the oxygen pressure beneath the sheet drops due to diffusion, bacterial action, etc., and the rate of oxidation to methemoglobin, i.e., the rate of discoloration is greatly increased. This assumption was checked by placing patches of vegetable parchment, which has a high oxygen permeability, on red meat. The area beneath the vegetable parchment did not discolor more rapidly than the uncovered surface.

The superiority of MSADT cellophane over MSAT may also be explained on the basis of increased oxygen permeability. When the exposed cellulose in MSADT cellophane became wet, its oxygen permeability would increase (6), maintaining the oxygen pressure and

thereby retarding methemoglobin formation. The development of an oxygen-permeable transparent sheet may solve the problem.

Summary

1. Discoloration in packaged red meats held at chill temperatures is not caused by only one specific wrapping material or by the coating on that material.

2. This discoloration is caused by the formation of a dark-brown substance known as methemoglobin. Methemoglobin is formed rapidly when the pressure of oxygen around the meat is reduced by the packaging material. Different packaging materials transmit oxygen at different rates; those with slower rates of transmission cause more rapid rates of discoloration.

3. The discoloration can be retarded by the use of suitable reducing agents.

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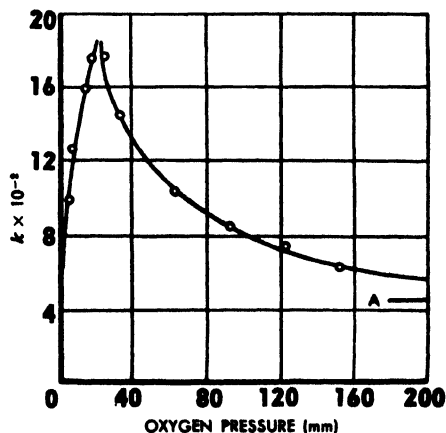
2. VELOCITY CONSTANTS (k), for the oxidation of hemoglobin to methemoglobin, at different oxygen pressure. A is the value at 723 millimeters of O₂.

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APPLES AS A SOURCE OF VITAMIN C[1]

E. P. GRANT

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APPLES AS A SOURCE OF VITAMIN C[1]

E. P. GRANT[2]

Experimental Farms Service, Ottawa, Canada

As early as 1912 interest was manifested in the Vitamin C content, or, as it was then known, the antiscorbutic factor, of apples. In that year Holst and Frolich (4) fed guinea pigs 30 gms. of raw apple daily to supply the antiscorbutic factor in the diet. Of four animals fed on the same diet three died on the 39th, 51st and 52nd days. The last two showed scurvy symptoms at post-mortem examination. The fourth animal was still alive after 87 days. The workers concluded that apples were not a good source of the antiscorbutic factor.

Bracewell, Hoyle and Zilva (1) tested a number of English apple varieties for the antiscorbutic factor and found Bramleys Seedling to be the best, being considerably above the others. In their test of a number of varieties of imported apples they found that antiscorbutic activity was greatest soon after harvesting.

In the study by Fellers, Isham and Smith (3) on the Vitamin C distribution in Baldwin and McIntosh apples the skin contained four times as much as the contiguous flesh and six to ten times as much as the flesh surrounding the core. McIntosh was low while Baldwin was a good source of Vitamin C.

Smith and Fellers (7) tested twenty-one varieties of apples grown in Massachusetts. Using guinea pigs as test animals they rated the varieties as follows:

Very Good—Baldwin, Northern Spy, Ben Davis, Winesap.

Good　　—Esopus, Rome Beauty, Red Astrachan, King Roxbury Russet, Rhode Island Stayman.

Fair　　—Arkansas, Gravenstein, Wealthy, Cortland, King David, Golden Delicious.

Poor　　—Jonathan, Delicious, Tolman, McIntosh.

The daily protective level for guinea pigs varied from 4 gms. for Baldwin to over 25 gms. for McIntosh.

Johansson (5) in Sweden found that Blenheim, Ecklinville and Wellington contained 20-25 gms. of Vitamin C per 100 gms. while Bramley, Golden Noble, Ontario and Transparente de Croncels had about 30 mgms. White Winter Calville was found to be much higher than all other varieties, the mean of three years' crops being 50 mgms.

The New York State Agricultural Experiment Station (6) have also reported on Calville Blanc. They found that for two years this variety averaged 37 mgms. per 100 gms.

In 1940 the writer did Vitamin C determinations on Canadian grown apples. The data obtained were not published at the time due to wartime difficulties. The indophenol titration method was used and peel and cortex were analysed separately. The following results were obtained:

TABLE 1

Variety	Average diameter	Peel	Cortex	Vitamin C per cored apple
	Inches	Mgms. Vit. C per 100 gms.	Mgms. Vit. C per 100 gms.	Mgms.
Wagener	2.25	44	12	19.9
Sandow	3	20.6	11.2	19.5
Donald	3	28.4	5.2	14.8
Wealthy	2.75	23	5.7	12.2
Northern Spy	2.5	20	7.0	12.0
Russet	2.75	16.3	6.9	11.1
Atlas	2.5	22.0	3.3	9.4
Rome Beauty	3	31.0	2.6	9.4
Linda	2.75	6.4	5.5	9.0
Cortland	3	14.7	3.7	8 5
Duchess	2.5	8.6	3 8	7.0
Baldwin	2 25	25.0	2.0	6 1
Gravenstein	2.75	6.4	2 8	4.9
Nonpareil	2 5	16.2	0.9	4 6
Joyce	3	4.8	1.9	4.5
Lobo	2 75	9.0	1 6	4.0
Cox Orange	2.5	4.6	2.8	3.7
Gano	2 75	12 6	0 9	3.4
Blenheim	3	6 4	0.9	2 9
Melba	2.5	2.9	1.9	2.7
Stark	2 75	7.7	0.9	2 6
Ribston	2 5	6.4	0.9	2 2
McIntosh	2.5	5.2	0 9	1.6
King	3	3 6	Trace	1.1

The Division of Chemistry, Science Service, Dominion Department of Agriculture (2) analysed 338 varieties and seedlings and of this number have found 19 to contain more than 15 mgms. of Vitamin C per 100 gms. of peeled and cored fruit. The following were high in this group:

Variety	Mgms. Vit. C per 100 gms.
Tony Crab	32
Red Tip Crab	27
Wapella	25
Gertrude	24
Transparent × Wealthy	21

From the above tables and the literature cited it can be seen that there is a wide variation in the Vitamin C content of different apple varieties. There are also many references in the literature, not quoted here, that bear out the same fact.

While some popular dessert varieties are quite low in Vitamin C content it is evident from the above figures that certain varieties of apples are a very good source of this vitamin. Thus, Wagener, Northern Spy and Russet which are popular dessert varieties are quite high in Vitamin C. As a comparison: oranges supply 35 to 55 mgms. of Vitamin C, per 100 gms., grapefruit 35 to 40 mgms. and tomatoes 15 to 30 mgms. per 100 gms. of fruit. Thus some of the varieties reported above compare very favourably with citrus fruits as a source of Vitamin C. In eating a "220" orange, a popular dessert size, one obtains about 55 gms. of Vitamin C; slightly more than this, or about 60 mgms. are obtained from eating three $2\frac{1}{4}$ inch Wagener apples.

The data given in Table 1 for Russet, Wagener and Northern Spy were obtained after these apples had been in storage about six months. These figures compare favourably with subsequent work done on fresh fruit of these varieties. This indicates that Vitamin C in apples is retained very well in storage. Johansson (5) also presents data to show that for the normal storage life of a variety there is good retention of Vitamin C.

Apples, besides their eye and taste appeal, are recognized as being very beneficial in human nutrition. They are a source of pectin, calcium, phosphorus and iron as well as Vitamins, A, B_1, G and C. With the stress that is at present being given vitamins it would be very desirable to have a good quality dessert apple that would be high in Vitamin C.

It is true that Calville Blanc and the varieties listed from the Division of Chemistry are not satisfactory eating apples. The New York State Agricultural Experiment Station in speaking of Calville Blanc say: "As an apple Calville Blanc can never be a popular kind even though high in Vitamin C. Few people will enjoy eating a fruit which is as unattractive in appearance and mediocre in quality as is Calville Blanc." This variety was at one time grown at the Experimental Farm at Ottawa but was long ago discarded as being an unsuitable variety.

By using varieties that are rich in Vitamin C even though poor in quality, plant breeders by breeding experiments and selection should be able to produce an apple that would be a very good source of Vitamin C and at the same time have the colour, flavour, etc., desired in a dessert apple.

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Comparative Value of Germicidal Ices for Fish Preservation

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ABSTRACT

The results obtained have shown that, in general, a germicidal ice which retards bacterial, spoilage of fish is much more effective when the pH of the flesh is low than when it approaches the neutral point. Of the ices used those containing 0.1% sodium nitrite, 0.01 to 0.02% sulphanilamide, 0.01% sulphathiazole and 0.067% chloramine B plus 0.067% sodium benzoate were quite effective in delaying spoilage. Ices containing chloramine B, chloramine T, No-Bac, Rivanol, penicillin, Roccal and Emulsept retarded spoilage only slightly or not at all.

Much of the literature dealing with results of experiments in which attempts had been made to retard bacterial spoilage of fish by icing them with ices containing germicides was reviewed previously (Tarr and Bailey 1939). A brief report concerning the present work has been published (Tarr 1946).

Germicidal ices might be divided into two classes: eutectic ices in which the added germicide is distributed uniformly through the ice, and non-eutectic ices in which distribution is uneven. Few eutectic germicidal ices appear to have been described. Heiss and Cürsiefen (1936-1937) prepared eutectic disodium phosphate ice (melting point about −0.9°C.) and eutectic sodium bicarbonate ice (m.p. about −2°C.), and found that fish iced with these ices did not spoil so rapidly as fish iced with ordinary ice. They attributed this finding to the fact that these ices maintained the fish at lower temperatures than did ordinary ice. The possibility that the rather alkaline solutions which formed when the ices melted exerted a mild bacteriostatic effect was apparently not considered. Bedford (Carter 1937, 1938 and unpublished) prepared an eutectic germicidal ice containing 0.16 per cent of benzoic acid. However, experiments showed that ice of this type effected no noticeable improvement in the bacteriological quality of iced fish, though it did specifically suppress trimethylamine formation (Tarr and Bailey 1939). Most of the germicidal ices which have been described have not been eutectic ices. In non-eutectic ices there is a strong tendency for the germicide to concentrate in the core of ice blocks during freezing, though a considerable quantity may be occluded throughout the ice mass, especially when solutions are not agitated (aerated) during freezing (Tarr and Sunderland 1940). Fine crushing and thorough mixing are necessary in order to obtain a fairly

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uniform distribution of germicide throughout the crushed ice in the case of non-eutectic ices. Probably modern methods of ice manufacture in which small quantities of ice are rapidly frozen from a solution (e.g. the Flak-ice method) could readily be adopted for preparation of such ices.

EXPERIMENTAL

The following germicides were used: alkyl dimethyl benzyl ammonium chloride (Roccal), N-acyl (colamino formyl methyl) pyridinium chloride (Emulsept), 2-ethoxy-6,9-diaminoacridine lactate (Rivanol), p-aminobenzenesulphonamide (sulphanilamide), 2-sulphanilyl-aminothiazole (sulphathiazole), sodium benzene-sulphonchloramide (chloramine B), sodium p-toluenesulphonchloramide (chloramine T), sodium penicillin, sodium benzoate, sodium nitrite and No-Bac (a commercial powder containing chloramine T and sodium benzoate). Frandsen (1946) has recently obtained a patent covering the manufacture of ice of this type under the trade name "Nipicide".

No attempt was made to prepare eutectic ices from any of these germicides. They were used in concentrations which would normally not increase excessively the cost of ice manufacture, which in aqueous solution were known in most instances to be actively bacteriostatic or bactericidal, and which would permit comparison with any available data from similar previous experiments. The ices were made either in commercial plants, the conditions of preparation being similar to those previously described (Tarr and Sunderland 1940), or by freezing about 15 kg. of solution in a galvanized iron container in still air at about $-28°C$. The ices were crushed, mixed and stored at about $-20°C$.

Analyses of approximately 2 kg. quantities of crushed ice were made in some cases in order to ascertain whether the added germicide was fairly well distributed. The following methods of analysis were used: that of the Rubicon Company (1939) for sulphanilamide, of White (1939) for sodium nitrite, and of Krog and Marshall (1940) for Roccal; and available chlorine in chloramine-containing solutions by the usual method involving liberation of iodine in acid potassium iodide solution, and titration of the iodine with standard sodium thiosulphate solution. The results of these analyses are given in brackets after the name and concentration of the compound used in preparing the ice (table I). They show that the recovery by analysis was lower than that expected from the quantity of germicide added in all except the 0.01% sulphanilamide ice, thus indicating an uneven distribution of the added chemical. Tests have shown that at 0°C. the solubility of sulphanilamide in water is about 0.2% and of sulphathiazole 0.015%.

The fish employed (table I) were, with the exception of the coho salmon, strictly fresh. They were obtained specially for the experiments and were iced, without being dressed, for less than a day before use. Most of the fish were still in rigor when they were iced in the experimental ices. The history of the coho salmon was not known. They were obtained from a local company and had been dressed, but not beheaded, and were stored in ice. They were only in fair condition and were typical of much of the fish of this type which is filleted. There

TABLE I. Comparative rate of spoilage of fish iced with various germicidal ices.

Species and composition of ice used	Init. pH of flesh	Bacteria in millions per g. of flesh after					
		5	7	9	11	14	17 days
Butter soles (*Isopsetta isolepis*)	6 0						
0.1% sodium nitrite (0.076%)		<0.1	<0.1	<0 1	<0 1	<0.1	<0 1
0.067% chloramine B (0.06%) + 0.067% sodium benzoate . . .		<0.1	<0.1	<0.1	<0.1	<0.1	1.4
0.0134% sulphanilamide.		<0.1	<0.1	<0.1	<0.1	0 21	0 27
0.067% chloramine B (0.064%)		<0.1	<0.1	<0 1	0.2	0.45	5.3
0.00067% Rivanol		<0.1	<0.1	<0 1	0 61	1.9	12.0
Water (control) .		<0.1	<0 1	<0 27	0 69	5 0	21

		5	7	10	12	14	17 days
Lemon soles (*Parophrys vetulus*)	6 2						
0.1% sodium nitrite (0.076%). .		<0.1	<0.1	<0.1	0 12	7 7	40
0.05% No-Bac.		<0.1	<0.1	1 2	18	240	*
0.01% Roccal (0.0084%)		<0.1	<0.1	<0 1	1.4	60	*
0.02% Roccal (0.0166%)		<0.1	<0.1	1.2	22	73	'
0.02% Emulsept.		<0.1	<0.1	1.1	29	53	*
Water (control)		<0.1	0 13	2 3	38	194	*

		4	7	9	11		days
Coho salmon (*Oncorhynchus kisutch*)	6.2						
0.1% sodium nitrite (0.088%). . .		<0.1	0.42	0.99	4.4		
0.01% sulphathiazole.		0.34	2.2	2.1	3.0		
0.01% sulphanilamide (0.01%). .		1 15	1.3	1 9	2.0		
0.2% chloramine B (0.193%) . . .		<0.1	0.86	2.0	1.7		
220 International units of penicillin per g.		0.64	2.1	3 6	2.5		
Water (control) . .		0.19	1.5	3 3	7 2		

		5	7	9	13	15	17 days
Starry flounder (*Platichthys stellatus*)	6 5						
0.1% sodium nitrite (0.088%). . .		<0.1	<0.1	0.16	0 21	0 33	3 0
0.01% sulphathiazole.		<0.1	<0.1	<0.1	<0 1	<0 1	1 0
0.01% sulphanilamide (0.01%). .		<0 1	<0 1	<0 1	<0.1	2 1	1 3
0.02% sulphanilamide.		<0.1	<0.1	<0 1	<0.1	0 14	3 2
220 International units of penicillin per g.		<0.1	<0.1	0.66	1 1	3 2	0.85
Water (control).		<0.1	<0 1	2 0	0.58	0 80	5.0

*These fish were putrid and no counts were made.

TABLE I. Comparative rate of spoilage of fish iced with various germicidal ices.

Species and composition of ice used	Init. pH of flesh	Bacteria in millions per g. of flesh after					
		4	6	8	11	13	15 days
Whiting (*Theragra chalcogramma*).......	6.5						
0.1% sodium nitrite (0.076%)...		<0.1	<0.1	<0.1	0.13	1.2	3.4
0.05% No-Bac.......... ...		<0.1	<0.1	<0.1	0.27	1.8	8.2
0.01% Roccal (0.0084%)..		<0.1	<0.1	0.27	2.2	9.8	29
0.02 Roccal (0.0166%)........		<0.1	<0.1	0.17	1.0	26	34
0.02 Emulsept.....		<0.1	<0.1	0.53	1.5	5.6	34
Water (control)......		<0.1	<0.1	0.45	2.1	2 4	19
Lingcod (*Ophiodon elongatus*)..........	6.6						
0.1% sodium nitrite (0.088%)...			0.32	0 74	1.1	16	
0.067% chloramine B (0.06%) + 0.067% sodium benzoate....			0.72	1.2	0.93	11	
0.0134% sulphanilamide...			<0.1	0.29	3.6	4.0	
0.067% chloramine T (0.060)...			0.16	0.82	3 0	12.0	
0.00067% Rivanol......... ...			2 4	2.2	3.7	7 7	
Water (control).			2.4	1 9	7 0	8 2	

was some variation in the method of treating the fish prior to icing. In order to simulate commercial practice neither butter soles nor starry flounders were washed or dressed before they were iced. Lingcod and whiting were dressed and the belly cavities washed well, but the external slime was not removed before icing. The coho salmon were re-iced without washing and the lemon soles were dressed, washed thoroughly and then iced. Four or six fish were iced with from 10 to 12 kg. of crushed ice in 27 × 27 × 38-cm. boxes, six boxes of fish being stored in each experiment in a well-insulated cabinet. It was found that under these conditions the ices melted slowly and that the air temperature was approximately 1.5°C.

The relative rates of bacterial spoilage of the iced fish were determined as follows. A single fish was removed from each box and, except with lemon soles, was washed well with running tap water. It was filleted on a clean board and the fillets skinned. Both fillets, or a representative portion thereof not exceeding 200 g., were ground to a paste in a Waring blendor, and water equivalent in weight to three times that of the ground fish was carefully stirred in so that incorporation of small bubbles was avoided. Direct bacterial counts (Tarr 1943) were made using this ground muscle suspension. This method is not accurate when there are less than about 100,000 bacteria per gram of flesh, unless a very large number of microscopic fields are counted. For this reason counts which were below this value were recorded as such. The initial pH of the mixed minced flesh of two

or three fish of each species investigated was determined using a Beckman pH meter. The results of these experiments are given in tables I and II.

Ice containing sodium nitrite was tested with each variety of fish for comparative purposes, because it had in previous experiments proven quite effective in retarding bacterial spoilage of four species of fish (Tarr and Sunderland 1940, 1941). The present experiments show that sodium nitrite ice markedly delayed spoilage of both butter and lemon soles in which the initial pH of the flesh was comparatively low. The fact that the coho salmon, which had a flesh pH value similar to that of lemon soles, were not nearly as effectively preserved by this ice, is probably explained by the fact that the fish were not very fresh at the commencement of the experiment, and that bacteria may have penetrated deeply into the muscle before the nitrite became sufficiently concentrated to exert a bacteriostatic effect. Sodium nitrite ice was considerably less effective in retarding bacterial spoilage of starry flounders and whiting than that of the lemon and butter soles in which the flesh was less acid, and only slightly delayed spoilage of lingcod which had the highest initial flesh pH value of any of the species investigated. Ice containing sulphanilamide markedly improved the keeping quality of butter soles, and also, though to a less marked extent, that of starry flounders. Ice of this type delayed bacterial spoilage of lingcod only slightly, and was ineffective in the case of coho salmon. Sulphathiazole ice effectively delayed spoilage of the starry flounders but not of the coho salmon. A slight improvement in the keeping quality of butter soles was occasioned by use of ice containing 0.067% chloramine B, but ice containing 0.2% of this compound did not delay the spoilage of the coho salmon. No improvement in the keeping quality of the lingcod was observed when chloramine T ice was used. Ice containing chloramine B plus sodium benzoate was more effective in retarding bacterial spoilage of butter soles than was ice containing only chloramine B, but it was practically ineffective with lingcod. No-Bac ice improved slightly the keeping quality of lemon soles and whiting, but was considerably less effective than sodium nitrite ice, especially with the lemon soles. Ices containing Roccal, Emulsept, sodium penicillin and Rivanol did not retard bacterial spoilage appreciably.

DISCUSSION

Although only one fish of each species has been examined at each time interval, the increase in number of bacteria in one type of ice is probably sufficiently consistent to justify the conclusions reached.

In general the results obtained show that ices of the type employed were not all effective, and those that were effective were most active when used on strictly fresh fish in which the initial flesh pH was quite low. Sodium benzoate (Cruess 1932), sodium nitrite (Tarr 1941), penicillic acid and 4-methoxy-2, 5 toluquinone (Tarr 1944) are most efficient as germicides when used in acid solution, and it is possible that this is true for certain of the germicides used in the present experiments. Probably the principal factors which determine the effectiveness of a germicidal ice in retarding bacterial spoilage of fish are the freshness of the fish used, the pH of its flesh and the ability of the germicide to penetrate unaltered

into the muscle. Thus sodium nitrite is quite effective because it diffuses into the flesh of iced fish (Tarr and Sunderland 1940, 1941) and is not affected by the flesh. It is not known whether sulphanilamide and sulphathiazole penetrate into the muscle of iced fish, but germicidal ices prepared from low concentrations of these compounds retarded bacterial spoilage quite markedly. Chloramines, though very powerful germicides, are readily inactivated in the presence of muscle tissue, and were not very effective in the present tests unless used in combination with sodium benzoate. In connection with the use of chloramines in ice it is of interest that a British patent covering the use of chloramine and dichloramine in ice was granted to DuPont de Nemours (1934). It has been shown that cationic detergent germicides are readily inactivated by phospholipids (Baker, Harrison and Miller 1941), and this may account for the ineffectiveness of ices containing Roccal and Emulsept in retarding bacterial spoilage. The lack of effectiveness of penicillin-containing ice might be due to the rather low concentration used or to the possibility that fish spoilage bacteria are not sensitive to this germicide. Rivanol ice was also ineffective, but the concentration used was very low. The results obtained with this compound are not in agreement with those of Keller (1940) who claimed that similar concentrations of Entozon in germicidal ice caused a very pronounced inhibition in the rate of bacterial spoilage of fish. Entozon contains Rivanol (29.44%), another acridine dye (5.88%) and sodium biborate (5.88%) as germicidal ingredients.

In conclusion it is desirable to point out that a germicidal ice may create a false impression of its true effectiveness by suppressing the formation of certain odoriferous substances associated with spoiling fish. Thus benzoic acid ice (Tarr and Bailey 1939) strongly suppressed trimethylamine formation in the flesh of iced fish without appreciably hindering the multiplication of bacteria.

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Special Ice-Salt Mixtures

May Lower

Refrigerator Car Temperatures

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THE first refrigerated railway cars used on this continent about 75 years ago' were cooled with ice or ice-salt mixtures placed in bunkers at each end of the car. The same basic principles have been in use ever since, despite the advances in refrigeration. This seemingly primitive procedure survives because it is economical, well suited to railway operation, and provides acceptable

Eutectic proportions of ice and salt yield minimum temperatures of —5 F in the bunkers of overhead iced railway cars, and car temperatures of 10 F or higher under summer conditions. Tests in a sectional model showed that the addition of 15 lb ammonium nitrate and 30 lb of sodium chloride per 100 lb. of ice yielded minimum bunker temperatures of —15 F. It is estimated that, by lowering the bunker temperatures 10 degrees in this manner, the car air temperature will be lowered 6-8 degrees. The mixing action produced by the motion of a car is essential for the maintenance of minimum temperatures with all salt-ice mixtures.

if not wholly satisfactory temperature conditions. The main faults are: first, large spatial temperature variations in the body of the car; and second, minimum air temperatures obtained are not always low enough for satisfactory transport of certain frozen perishables Canadian railways have reduced the first defect to an acceptable level by placing the bunkers in the roof, over the load, instead of at the ends of the car [9] [10] [12] The second defect arises from the use of ice and sodium chloride as the cooling medium having a eutectic temperature of --6 F. The lowest bunker temperature attained with such mixtures in refrigerator cars is -5 F. and under summer conditions this yields a car air temperature of 10 F or higher. At present there is a growing demand for transport temperatures approaching 0 F. The object of the present investigation was to obtain a cooling mixture based on ice that would yield lower temperatures than ice and sodium chloride when used in overhead iced cars.

Use of modern refrigeration equipment has been proposed to provide lower and more satisfactory temperature conditions in railway refrigerator cars. Some of

this equipment has been tested or used to a limited extent commercially [4, 5, 6, 11, 13, 14]. In spite of this, ice-salt mixtures have survived on this continent largely on economic grounds. In general, the proposed equipment, even if satisfactory from a refrigeration standpoint, would increase unit capital and maintenance costs and consequently would increase the rates. Capital costs for refrigeration equipment must be kept low on Canadian railways as their earning season is short. Refrigeration is not generally required during the winter months and even in summer the movement of perishables is frequently a one-way traffic.

Mechanically refrigerated cars would need special mechanical crews at divisional points. Modified absorption systems[4] require ammonia recovery equipment. The conversion of such "special" cars for the handling of perishables would introduce additional problems, such as rugged, reliable thermostats unaffected by shunting. Eutectic ices [1, 2] demand low temperature freezing facilities and, to provide a range of temperatures, various eutectic mixtures would be needed. Dry ice [8, 11] is costly and difficult to distribute and preserve at icing stations. Since the overhead iced car is satisfactory for most present day needs, these newer methods of refrigeration cannot be justified on economic grounds until the volume of low temperature traffic is sufficient to bear the extra cost.

The immediate Canadian problem is therefore one of lowering the temperature range obtained in the ice cooled overhead bunker car. Three general methods might be used for obtaining lower temperatures: (1) a lower temperature cooling medium might be employed; (2) the cooling surface might be increased to reduce the temperature difference between the cooling medium and the air; or (3) the insulation on the car might be increased to reduce heat gain. Increasing the insulation on the car would increase the capital cost, and would be extremely difficult to apply to existing equipment. Increasing the amount of cooling surface or other internal alterations would present the same problems, although to a lesser extent, since only part of the car would be affected. It is doubtful, however, if any practical means for increasing the cooling surface alone would be sufficient to obtain the desired low temperatures. In these circumstances first attention was given to the possibilities of obtaining a cooling medium at a lower temperature.

Substitution of Salts Other Than NaCl

The possibility of using salts other than sodium chloride with ice to obtain lower temperatures was first assessed. Since railway cars have been cooled with ice plus sodium chloride in varying proportions, it appears that a eutectic mixture is not essential. It is, however, obvious that the eutectic temperature of any alternative salt must be lower than that of sodium chloride and ice. A considerable number of salts having lower eutectic temperatures exist and a number of these have been used successfully as prefrozen eutectic ices in other refrigeration applications [1, 2].

However, mixing dry salt with pure ice in the required proportions differs both economically and technically from the use of a prefrozen eutectic ice. Many salts are too costly for railway car applications unless
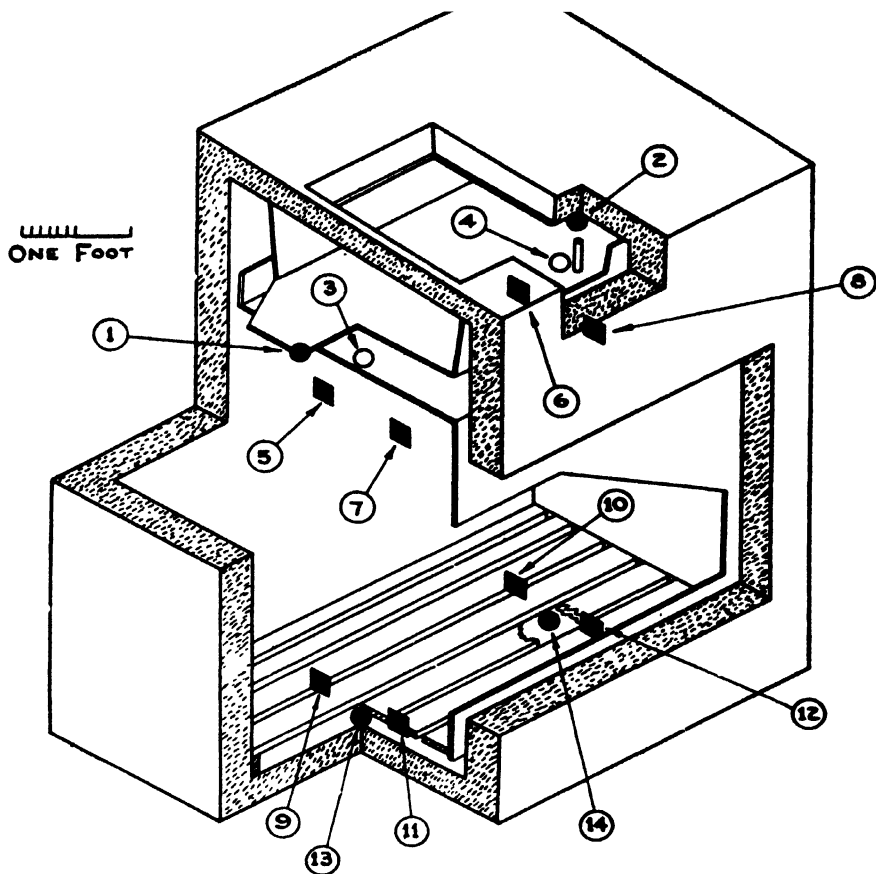
Fig. 1. Schematic diagram of model railway refrigerator car used in tests.

the solutions are recovered and refrozen and this procedure is precluded on economic grounds.

The refrigerating effect of salt-ice mixtures is less than that of a prefrozen eutectic ice, since ice is melted to cool the components and solution to the desired temperature and also to absorb the heats of solution and hydration of certain salts. As an extreme example, the addition of sufficient anhydrous calcium chloride to a pound of ice to produce temperatures in the region of —20 F would yield a mixture capable of absorbing only about one-tenth as much heat as a similar amount of ice and sodium chloride in eutectic proportions, since in the former most of the ice would be melted to overcome the heat of hydration. On the other hand, the use of pre-hydrated calcium chloride would demand such a high proportion of salt that the ice carrying capacity of the tanks would be substantially reduced.

An examination of the available information, including the well known laboratory freezing mixtures, eliminated all the salts considered for one or more of the following reasons: supply limitations, cost, proportion of salt required, or lack of adequate refrigerating effect when mixed with ice. The possibility of substituting another salt for sodium chloride appeared therefore to be remote.

Freezing Mixtures of Ice Plus Two Salts

Attention was then turned to the possibility of depressing the eutectic temperature of sodium chloride-ice mixtures by the addition of a second salt. This alternative appeared to have attractive possibilities. If the second salt was required in relatively small proportions, the increased cost might not be prohibitive. From the technical standpoint the possibility of using another salt in small proportions would have less effect in reducing the refrigerating capacity through heat contributed by hydration or solution. It also increased the number of potentially useful salts since those having eutectic temperatures above the desired range might produce the required temperatures when mixed with sodium chloride.

The first point was to demonstrate that the freezing point of sodium chloride-ice mixtures is approximately eutectic proportions could be reduced by the addition of a reasonable proportion of a second salt. Mazzotto's results on the freezing point of mixed solutions containing sodium chloride indicated that the desired objective could be attained. Some of his data appear in the first part of Table 1. These results indicate that the melting points of certain salt mixtures containing sodium chloride are lower than the eutectic temperature of sodium chloride alone. In some instances, however, this depression in the melting point was not sufficient to be of practical value, and for others the amount of the second salt required was too high to be economically feasible.

Some preliminary experiments were undertaken to determine the melting and freezing points of solutions made up by adding 30 parts of sodium chloride and 10 parts of other salts to 100 parts of water. These solutions were placed in containers fitted with a thermocouple, and the freezing and melting zones were determined. The results of some of these preliminary observations appear in the second part of Table 1. They indicate that the freezing and melting points of these solutions were lower than the eutectic temperature for sodium chloride. The addition of potassium chloride and sodium nitrate did not depress the freezing points sufficiently to be of practical value. The addition of calcium chloride and sodium hydroxide, while effective for depressing the eutectic temperature, particularly when present in higher proportions than those reported in Table 1, would be of little value owing to their high heats of hydration and solution. The mixtures employing ammonium chloride and ammonium nitrate showed promise for depressing freezing point below eutectic temperature of sodium chloride solutions.

Although these results indicated that the freezing and melting points could be depressed, they gave no indication of the temperatures that would be obtained when the solid salts were added to ice in the same proportions. Tests were made by adding the requisite amount of the salts to ice in an insulated container, fitted with a thermocouple, and shaken during the period of observation. The minimum temperatures attained were considerably above the freezing points of solutions of the same composition. The temperatures observed were dependent on

Table 1. Freezing Point of Mixed Solutions Containing Sodium Chloride

Salts	Amount of salt per 100 lb water, lb	Freezing point, F
MAZZOTTO'S DATA		
NaCl	25.6	—10 6
KCl	8.7	
NaCl	20.6	11.4
NaNO₃	31 0	
NaCl	25.0	13.0
NH₄Cl	10.3	
NaCl	27.8	—22.2
NH₄NO₃	58.3	
PRELIMINARY TESTS BY AUTHORS		
NaCl	30.0	—11.0
CaCl₂	10.0	
NaCl	30.0	—13.0
KCl	10.0	
NaCl	30.0	—13.0 or lower
NaNO₃	10.0	
NaCl	30.0	-14.5
NH₄Cl	10.0	
NaCl	30.0	20 0
NaOH	10.0	
NaCl	30.0	—22.0
NH₄NO₃	10.0	

the size and rate of heat uptake of the test vessel as well as on composition of the mixture. The results of these tests were useful for preliminary evaluation of new mixtures but, as they gave little information to be expected in practice, the results are not reported.

Experiments with Model Car

These preliminary experiments showed that subsequent tests would have to be made with equipment approximating the scale and rate of heat gain that would take place in a railway car. A modified half-scale model representing one bunker area or one-eighth of a car was constructed and used for all subsequent tests.

Description of Model—The model shown in Figure 1 was made from angle and galvanized iron. It was about 5 ft long, 5 ft high and 3 ft wide. The bunker held 150 lb of crushed ice and was 52 in. long, 23 in. wide and averaged 8 in. in depth. An overflow pipe retained a 6-in. depth of brine. The depths of the bunker and brine were essentially those of full scale equipment. While at least two of the four walls of the model would represent "internal" surface in a full scale car, these were not given any special treatment, all surfaces being insulated with 4 in. of Ten-test or equivalent insulation.

From the thicknesses of insulation used, the heat conductivity of the model was estimated at about 0.1 Btu per sq ft per hr per degree F. Toward the end of the tests reported here, the heat conductivity was determined by cooling the room to 40 F and heating the interior to several "steady" temperatures with known electrical inputs. From the observed temperatures, heat input, and dimensions, the conductivity was found to be 0.12 Btu per sq ft per hr per degree F.

Since the motion of a railway car contributes a stirring action, the base was mounted on a shaft that permitted the model to be oscillated from side to side at a

rate of 29 cycles per minute, the movement of the bunker being about 3½ in. A drip tray was installed beneath the bunker and extended into a false wall along one side of the car to simulate the standard air circulation system. A hatch in the roof and a door to the air chamber provided the necessary openings for inspection, charging, and thermocouple installation.

Method of Testing—An important condition was to obtain about the same rate of heat transfer between the bunker and the air space as existed in railway cars. An examination of available test data with overhead-iced cars indicated that when maximum refrigeration was provided under severe summer conditions, the air temperature in the car might be 20 to 25 F higher than the estimated temperature of the bunker mixture. To attain this condition the model was placed in a controlled temperature room at an initial temperature of about 35 F. Salt and ice in eutectic proportions yielded a bunker temperature of approximately —5 F with the model in motion. The temperature in the room was then increased until the average car air temperature reached 17 F or a temperature differential of 22 degrees between bunker and car air. This occurred when the temperature in the test room reached 60 F and all subsequent tests were conducted at that temperature. Ordinarily a differential of 22 degrees between the bunker and air temperatures would not occur in a railway car at ambient temperatures of 60 F, but the greater surface-volume ratio of the small sectional model could readily account for the higher differential occurring at lower ambient temperatures.

The test procedure consisted of charging the bunker to capacity with crushed ice (sizes to pass 2 in. sq grid) and one or more salts in the required proportions. The additions of the several components were usually made batchwise, and they were mixed to some degree while being forced back in the bunker—a procedure comparable with railway practice. All parts of the model were at temperatures above the freezing point at the start of each test to insure that the lower surface of the bunker was not coated with frost. After a precooling period of six to eight hours, the bunker was recharged to capacity with ice and salt in the required proportions. The amount of ice required varied with temperature conditions but was usually from 40 to 100 lb.

Periodic readings were taken on the thermocouples during the precooling period. When all temperatures reached a steady condition, readings were taken every 45 minutes. An average of four or more consecutive readings that were consistent, within errors of measurement, was taken as the minimum temperature obtainable for a given condition or icing mixture.

The position of the thermocouples is indicated in Figure 1. Thermocouples 1 and 2 were placed in the freezing mixture in the bunkers and the results were averaged to obtain the bunker temperatures reported. These observations on bunker temperatures were frequently supplemented by thermometer readings. Thermocouples 3 and 4 were placed in the space between the bunker and drip tray. The results obtained with the eight thermocouples in the main air chamber (5 to 12 inclusive) were averaged and reported as the air temperature. Thermocouples 13 and 14 were beneath the floor racks. The

Table 2. Temperatures Attained in Freezing
Mixtures in Model Bunker

Salt mixture	Amount of salt per 100 lb ice, lb	Temperature attained, F
NaCl	30	— 6.0
KCl	10	
NaCl	30	— 6.0
KCl	15	
NaCl	30	— 8.5
NH₄Cl	10	
NaCl	35	— 9.0
NH₄Cl	15	
NaCl	35	— 7.4
NH₄Cl	20	
NaCl	30	— 8.0
NaNO₃	15	
NaCl	30	—10.0
NaNO₃	20	
NaCl	30	—10.0
NaNO₃	25	
NaCl	30	—15.0
NH₄NO₃	10	
NaCl	30	—15.0
NH₄NO₃	15	

location of additional thermocouples placed behind the
false wall to indicate the direction of air flow is not
shown as these measurements were considered of little
value and are not reported.

Results

The results obtained when the four most promising
salts were added to sodium chloride-ice mixtures in
eutectic proportions appear in Table 2. The addition of
potassium chloride yielded essentially the same mini-
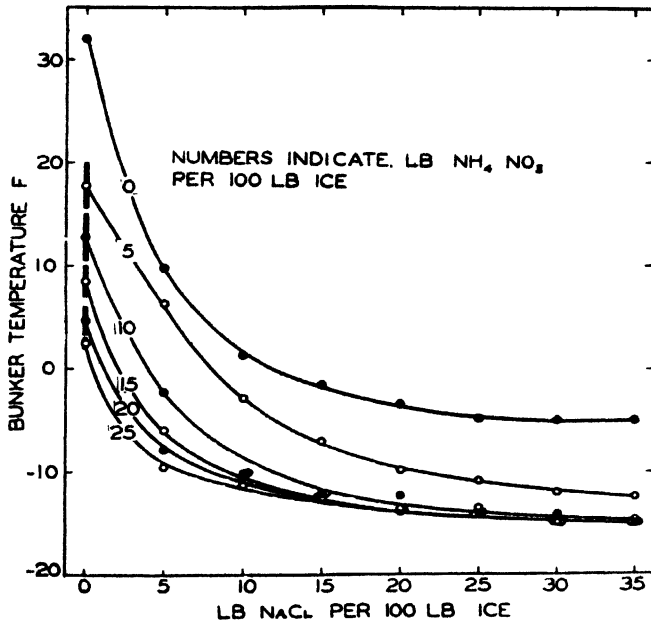
Fig. 2. Bunker temperatures with ammonium nitrate-sodium
chloride ice mixtures.

mum temperatures as sodium chloride alone, while ammonium chloride and sodium nitrate depressed the temperature only a few degrees below that of the conventional ice-salt mixture. Ammonium nitrate, however, when added in reasonable proportions, lowered the minimum temperature to —15 F or about 10 degrees below that obtained with ice and sodium chloride. In consequence all further work described here was confined to a more intensive study of ice, sodium chloride, and ammonium nitrate mixtures.

The results of extensive trials on sodium chloride, ammonium nitrate, and ice mixtures appear in Figure 2. The upper curve shows the temperatures obtained when sodium chloride alone is added to ice in various proportions. A relatively small amount of this salt causes a substantial decrease in temperature but succeeding increments become progressively less effective. Temperatures of 0 F were attained with less than 15 lb of sodium chloride per 100 lb of ice, but 30 lb of salt only yielded temperatures of about —5 F. The latter amount is essentially the eutectic concentration (30.4) and no lower temperature was observed with 35 lb. The temperature reductions at increasing salt concentrations, above 20 per cent, were small and, except for providing a reserve, would contribute little in practical operations.

Addition of ammonium nitrate to ice in various proportions did not lower the temperature to the same extent as similar additions of sodium chloride. Furthermore, the observed temperatures were much more variable than those obtained with sodium chloride or sodium chloride-ammonium nitrate mixtures. It appears that ammonium nitrate dissolves far more rapidly than sodium chloride. This action tends to melt a portion of the ice and produce a brine approaching eutectic concentration, yielding a temperature below that to be expected for the quantity of ammonium nitrate added. After a short period, the temperature starts to rise as more ice is melted and the salt concentration is reduced. This instability is indicated by the lines between the points on the lefthand side of Figure 2. Nevertheless, it is evident that the addition of ammonium nitrate to ice did not produce as low temperatures as the addition of corresponding quantities of sodium chloride. This behavior was to be expected from the higher eutectic temperature and higher eutectic concentration of ammonium nitrate as compared with sodium chloride.

The addition of ammonium nitrate to sodium chloride-ice mixtures reduced the temperatures at all sodium chloride concentrations. The addition of 5 lb of ammonium nitrate per 100 lb of ice had less effect at low than at high sodium chloride concentrations. Adding 10 lb of ammonium nitrate yielded almost the full temperature depression observed at all sodium chloride concentrations above 10 lb per 100 lb of ice. Higher concentrations of ammonium nitrate produced only small temperature decrements. It is evident from Figure 2 that the addition of 25 lb of sodium chloride and half that quantity of ammonium nitrate will yield freezing mixtures within a degree or so of the minimum attainable. The use of still higher concentrations of both salts to obtain only slightly lower temperatures cannot be justified on economic grounds, although operations may indicate some excess of both salts is desirable.

The results of the air temperature measurements are

Table 3. Effect of Motion on Bunker Temperature

Cooling mixture, lb per 100 lb ice	No. of tests	Bunker temperature, F			Final bunker temperatures*, F		
		In motion	Stationary	Difference	After extended shaking	On resumption of shaking	Difference
25 sodium chloride	2	— 5.0	4.1	9.1	8.2	0.2	8 0
30 " "	3	— 5.0	2.9	7.9	3.8	— 3 6	7 4
15 ammonium nitrate	1	—12.5	18.3	5.8	—	-	—
30 " " 15 sodium chloride	1	2.5	7 1	4.6	—	- -	
15 ammonium nitrate 30 sodium chloride	1	12 5	0.6	13.0	--	- -	. .
10 ammonium nitrate 30 sodium chloride	1	—14.0	—6.0	8.0	—	-	—
15 ammonium nitrate	3	—15.0	9'9—	8.4	—1.5	—12 2	10.7
Avg. increase when standing (individual test runs)				8.3			8 7

* Results of single tests

summarized in Figure 3 where they are plotted against the corresponding bunker temperature. A 45 degree reference line has been indicated in this figure, since the distance between it and the experimental curves shows the effective temperature difference in the model at different bunker temperatures. Since the model was operated in a room at 60 F, it is obvious that the experimental and 45° lines must coincide at this point.

The upper curve shows the average temperature observed on the eight thermocouples distributed through the air space and the two thermocouples beneath the floor racks. The latter thermocouples indicated temperatures essentially the same as those in the space above. Readings from the two thermocouples placed between the bunker and drip pan were averaged and the results are given as "temperature above drip pan" in Figure 3 They showed slightly lower temperatures than those in the air space below. Little significance can be attached to this observation made in the model, except that it suggests that the drip pan tends to increase the overall temperature difference between the bunker and the air.

When the insulation and heat transfer surfaces are fixed, the bunker and ambient air temperatures will be the primary factors determining the temperature in the car. The slope of the car air temperature curve in Figure 3 shows a 6.6 degree reduction for each 10 degree reduction in bunker temperature. In other words, the overall temperature difference betwen the bunker and ambient air is divided one-third bunker-to-car air and two-thirds between the car and ambient air temperatures. Since the scale and design of the model differ substantially from a regular car, these figures may not be representative of what occurs in practice. The results of some railway operating tests, to be reported in detail later, were examined to determine the air temperatures to be expected. In three tests at average outside shade temperatures of about 66 F and bunker temperatures of about —3 F, the car temperature corresponded to a reduction of 7.7, 7.8, and 7.9 degrees for each 10 degrees reduction in bunker temperature below the outside air Under similar conditions bunker temperatures of about —10 F resulted in air temperatures corresponding to a reduction of 7.6 and 7.8 degrees for each 10 degrees reduction in bunker temperature. It appears, therefore,

that a 10 degree reduction in bunker temperature, within the temperature range under consideration, will reduce the air temperature in the car about 7 to 8 degrees.

Effect of Motion on Bunker Temperature—Observations by railway personnel indicated that overhead iced cars, employing identical ice-salt mixtures, cooled more rapidly when the cars were moving than standing still. This suggested that mixing was essential for the maintenance of minimum bunker temperatures. The degree of shaking provided for the test model was obviously adequate since salt-ice mixtures in eutectic proportions yielded temperatures of approximately —5 F. Comparable tests were therefore made with the model stationary to establish the magnitude of the temperature differential and also to determine the effect of adding ammonium nitrate on this differential.

The results of these tests are given in Table 3. The reported temperatures with the bunker in motion were taken from the data used to construct Figure 2. Identical mixtures in the stationary model gave the results reported in the next column. Such observations are
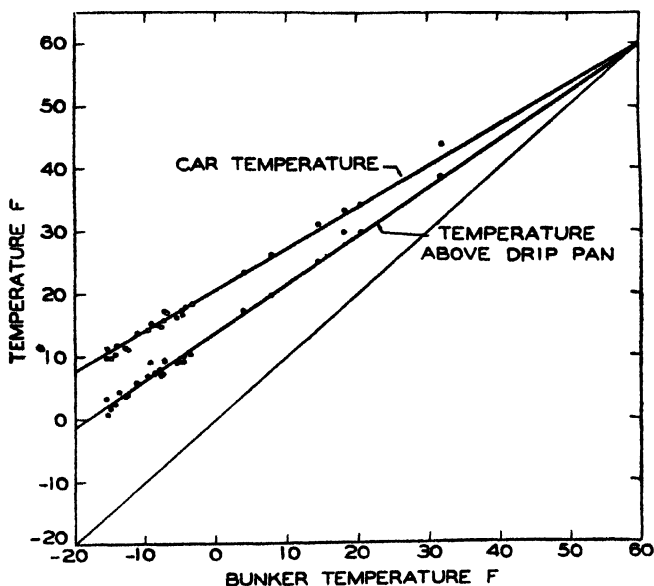

Fig. 3. Temperature measurements in model refrigerator car.

subject to considerable variability but on the average the temperatures observed with the model stationary were 8 degrees higher than those for similar mixtures with the model in motion. The effect of motion appears to be somewhat less for ice-ammonium nitrate mixtures, probably because this salt dissolves more rapidly.

To determine if this effect would persist as the ice melted and the proportion of brine increased, the more important mixtures were allowed to stand until the bunker temperatures had risen several degrees above the steady initial values. The temperatures were then read, the bunker set in motion, and the temperatures taken again when they became steady. The results appear in the last part of Table 3. Even in these partly spent mixtures motion reduced the temperature of the mixtures more than 8 degrees.

These results indicate that motion is essential to obtain minimum temperatures with ice-salt mixtures. Cars that are not in motion will give bunker temperatures about 8 degrees above the minimum. Motion therefore lowers the temperature to an extent almost equal to the addition of ammonium nitrate. Since loaded cars are usually moving, this observation has little general significance but it may attain importance if loaded cars are held stationary for lengthy periods.

Summary

The foregoing results indicate that the addition of ammonium nitrate to the usual sodium chloride-ice mixtures should reduce bunker temperatures about 10 degrees and car temperatures 6 to 8 degrees. There would not appear to be any practical difficulties in the use of ammonium nitrate since its use involves essentially the same facilities as current procedures. This method is also applicable to existing cars and need only be used where maximum refrigeration is required. Additional charges will be necessary to cover the cost of ammonium nitrate, additional ice consumed, and incidental operating expenses, but this charge would not appear prohibitive for shipments requiring maximum protection. In these circumstances practical operating trials were undertaken to assess the value of ammonium nitrate additions in railway practice. The results of these trials will be reported in detail later.

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CANADIAN WILTSHIRE BACON

XXX. EFFECTS OF CURING AND COOKING ON THE THIAMINE, RIBOFLAVIN, AND NIACIN CONTENTS OF LONGISSIMUS DORSI MUSCLES

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CANADIAN WILTSHIRE BACON

XXX. EFFECTS OF CURING AND COOKING ON THE THIAMINE, RIBOFLAVIN, AND NIACIN CONTENTS OF LONGISSIMUS DORSI MUSCLES[1]

By Dyson Rose[2] and Ruth Peterson[2]

Abstract

Laboratory studies on the thiamine, riboflavin, and niacin contents of longissimus dorsi muscles and of spent pickle indicate that there was little or no destruction of these vitamins during the curing process but that approximately 24% of the thiamine, 12% of the riboflavin, and 29% of the niacin were leached from the meat. The losses due to leaching increase with greater relative pickle volumes and are greater from an exposed surface of lean meat than from a surface covered with fat or rind. The commercial practice of rebuilding spent pickle probably reduces the over-all vitamin losses.

The losses of thiamine and of riboflavin incurred during the cooking of pork were found to be considerably greater than those incurred during the cooking of bacon. Niacin was lost to about an equal extent from both pork and bacon. The stabilization of the thiamine and riboflavin that occurred during the curing process was sufficient to offset the losses during cure and thus, after cooking, bacon was as good a source of these vitamins as pork.

Introduction

The B-vitamin content of pork has been estimated in several laboratories but the losses incurred during the conversion to bacon have not been studied extensively. Jackson and his coworkers (4) determined the retention of thiamine, riboflavin, and niacin following "wet cure" of bacon and found a loss of thiamine, niacin, and probably riboflavin, but the loss varied considerably from sample to sample. McIntire *et al.* (5) report values for the thiamine and niacin contents of "Canadian bacon" that are somewhat below the usual values for fresh pork, indicating that a loss may have occurred during processing. Schweigert and coworkers (11) have also reported losses of thiamine, riboflavin, and niacin from pork hams during cure. Working in these laboratories, Gorham (2) found lower thiamine, and niacin contents of Wiltshire half-backs after curing in pickle but found no significant change in the riboflavin content.

The results of numerous studies on the effect of cooking on the vitamin content of pork and pork products have indicated that 10 to 50% of the thiamine, 10 to 25% of the riboflavin and 0 to 20% of the niacin may be lost (1, 2, 9). Variations in the losses have been related to differences in the type of product studied and in the time, temperature, and method of cooking (4, 7, 8, 11). The studies of Rice *et al.* (8), of Nymon (6), and of Gorham (2) have indicated that the stability of these vitamins may be influenced by the

admixture of other materials into the pork products and by the curing processes. The work of Gorham (2) appeared particularly interesting as it indicated that a change occurring during the curing of Wiltshire bacon stabilized the niacin against subsequent loss during cooking.

The studies reported in the present paper were undertaken to determine the fate of the vitamin lost from the meat, the effect of variations in the Wiltshire curing process on the extent of the loss, and the effect of commercial curing on the vitamin losses during subsequent cooking.

Analytical Methods

The methods used for estimating the vitamin content of the lean meat were essentially the same as those previously described (2), thiamine by the thiochrome procedure, riboflavin by a fluorometric method, and niacin by microbiological assay. At first, pickle samples were assayed without preliminary hydrolysis but it was found that hydrolysis increased the thiamine assay values, and an enzymic hydrolysis of a 1 : 10 dilution of the spent pickle was made for all subsequent thiamine assays. For convenience, niacin assays were also made on the hydrolyzed samples although the hydrolysis had no significant effect on the results. The 10-fold dilution, which was necessary to avoid salt inhibition of the hydrolytic enzymes, was too great to permit subsequent riboflavin assay. Hydrolysis with sulphuric acid was tried but the assays were of low accuracy owing to the high salt (sodium chloride plus sodium sulphate) concentration. All pickle samples were therefore assayed for riboflavin directly by a microbiological procedure, with sufficient dilution to avoid serious interference from the sodium chloride. The technique used was essentially that of Snell and Strong (12), but the basal medium was modified by doubling the yeast extract, tripling the dextrose, and adding 6 gm. of crystalline sodium acetate and 20 ml. of casein hydrolyzate (General Biochemicals Ltd.) per 500 ml. of basal medium.

Moisture and chloride determinations were made on the lean meat samples by methods previously described (3). The results of analysis of variance of the dry weight data did not differ essentially from those of the moist weight data, so the assay values, with the exception of those for the cooking experiment, are reported as μgm. per gm. of fresh or cured lean meat. For comparison the vitamin content of the pickle is also reported as μgm. per gm. of meat, the conversion from μgm. per ml. of pickle being made by multiplying by the pickle : meat ratio.

Experimental

Variability in Materials

Rice *et al.* (9) and others have shown that a considerable variation in vitamin content may occur from muscle to muscle in a single hog as well as in the same muscle from different hogs. In the present work the variability was reduced as much as possible by making all determinations on the longissimus dorsi muscles. Marked variations were found even within this muscle (Table I) although the variation between samples from one hog was less than

TABLE I

VARIATIONS IN VITAMIN CONTENTS (μGM. PER GM.) OF LONGISSIMUS DORSI MUSCLES
IN FRESH PORK

Vitamin	Between 11 different hogs		Between 10 samples from the same hog	
	Range	Average	Range	Average
Thiamine	8.1 – 15.4	12.3 ± 2.1	13.2 – 13.8	13.5 ± 0.2
Riboflavin	1.30 – 1.76	1.50 ± 0.15	1.54 – 1.84	1.65 ± 0.11
Niacin	26.7 – 78.7	53.5 ± 4.5	35.3 – 42.2	40.9 ± 2.1

that between hogs. The data indicated a slight decrease in the vitamin content from the fore end to the back of the hog but the differences were significant (5% point) for riboflavin only.

Effect of Salt Concentration and Duration of Cure

To determine the effects of brine concentration and pickling time random sections of longissimus dorsi muscles weighing approximately 200 gm. each were immersed individually in brines containing 1% sodium nitrate, 0.05% sodium nitrite, and 15, 22.5, or 30% sodium chloride. Curing times of zero to seven days at 40° F. and a meat : pickle ratio of 4 : 3 (by volume) were used. All samples were ground shortly after removal from the pickles and were stored at −10° F. until analyzed. The spent pickle was stored at 30° F. until analyzed (about six weeks); prolonged storage did not significantly affect the assay values. The experiment was conducted in triplicate. Thiamine, riboflavin, and niacin were determined on the meat and spent pickle, and chloride and moisture determinations were made on the meat.

The chloride content of the meat increased with curing time and with pickle concentration, the final (seven day) values averaging 5.72, 7.25, and 9.02% for the meat from the 15, 22.5, and 30% brines respectively. Moisture content of the meat decreased with time and with brine concentration, being 73.1% in the fresh meat and 71.1, 69.4, and 67.5% after seven days cure in the 15, 22.5, and 30% brines.

Table II shows the average thiamine, riboflavin, and niacin contents of the meat and spent pickle together with the necessary differences for significance. The salt concentration did not significantly affect the distribution of thiamine or niacin but the amount of riboflavin found in the pickle increased with increasing salt concentration (average values 0.10, 0.13, 0.14 μgm. per gm. for the 15, 22.5, and 30% pickles respectively; necessary difference for significance, 5% level, 0.015).

These data show that although only the thiamine and niacin content of the meat decreased significantly during cure, all three vitamins accumulated in the pickle. Riboflavin, as well as thiamine and niacin, must therefore have been lost from the meat but the losses involved were small relative to the

TABLE II

DISTRIBUTION OF THIAMINE, RIBOFLAVIN, AND NIACIN (μGM. PER GM OF MEAT) IN PORK
AND PICKLE DURING CURE

Days in pickle	Pickle	Meat	Total
Thiamine			
0	—	13.2	13.2
1	1.2	11.3	12.4
2	1.6	9.9	11.4
3	2.2	9.8	12.0
4	2.5	9.7	12.1
5	2.7	9.1	11.9
6	3.2	9.2	12.4
7	3.2	9.2	12.4
Necessary difference[a]	0.46	1.49	—
Riboflavin			
0	—	1.46	1.46
1	0.06	1.36	1.42
2	0.09	1.39	1.48
3	0.12	1.36	1.47
4	0.14	1.46	1.60
5	0.13	1.23	1.36
6	0.16	1.31	1.47
7	0.17	1.40	1.57
Necessary difference[a]	0.023	—	—
Niacin			
0	—	57.3	57.3
1	7.9	41.6	49.5
2	9.4	36.0	45.4
3	11.4	36.1	47.5
4	12.5	38.0	50.5
5	15.7	41.9	57.7
6	16.6	38.0	54.6
7	15.7	34.3	50.0
Necessary difference[a]	3.6	11.0	—

[a] Necessary difference for significance, 5% point.

variability between samples and are thus masked when the assay values for
the meat are considered. The average amount of vitamin in the pickle at the
end of the seven day cure represented 24.2% of the thiamine, 11.8% of the
riboflavin, and 29.0% of the niacin originally present in the meat. Since the
total amount of each vitamin remained constant within the limits of experi-
mental error, these figures also represent the approximate percentage loss from
the meat under these conditions.

Effect of Rind and Fatty Tissue.

Three samples of spent pickle obtained from a commercial packer were
assayed and were found to contain 1.1 μgm. of thiamine, 0.06 μgm. of ribo-
flavin, and 3.1 μgm. of niacin per gram of meat cured. These results are
lower than those obtained in the laboratory work and it appeared probable that
the greater area of lean and cut surface exposed to the pickle during the
laboratory cure had allowed a greater amount of vitamin to leach from the

meat.   To test this assumption all but one of the lean surfaces of 10-in. central sections from the right sides of three hogs were covered with lard and the sections were immersed in 30% brine.   At the end of a seven day curing period at 40° F. the sections were sliced longitudinally and the slices of longissimus dorsi, trimmed free of fat, were apportioned so as to give four samples of approximately 125 gm. each taken at increasing depths below the exposed surface.   The left sides from the same hogs were similarly sliced when fresh to serve as controls.

The losses, expressed as average differences between the corresponding control and cured samples, are given in Table III.   The loss of thiamine and niacin decreased markedly as the distance from the exposed surface increased, but the losses of riboflavin were too small to show significant variations.

TABLE III

AVERAGE LOSSES OF THIAMINE, RIBOFLAVIN, AND NIACIN FROM CURED PORK SAMPLED
AT VARIOUS DEPTHS BELOW A SINGLE EXPOSED SURFACE

(Averages for three hogs, μgm. per gm. of meat)

Approx. depth, in.	Thiamine	Riboflavin	Niacin
0 – 0.4	5.6	0.07	34.0
0.4 – 0.8	2.2	−0.10	21.7
0.8 – 1.2	0.7	−0.10	10.1
1.2 – 1.6	1.0	0.03	7.5
Necessary difference, 5% point	1.31	—	9.88

Effect of Meat : Pickle Ratio

Six random sections of the longissimus dorsi muscles from each of three hogs were used to determine the effect of increasing the volume of brine relative to the meat.   A brine of 1.0% sodium nitrate, 0.05% sodium nitrite, and 30% sodium chloride was used at meat : pickle ratios of 1.0 : 0.5, 1.0 : 0.8, 1.0 : 1.0, 1.0 : 1.3, and 1.0 : 2.0 by volume.   The samples were cured individually for seven days and were then ground and stored at 40° F. until analyzed.

The thiamine, riboflavin, and niacin contents are given in Table IV.   Significant quantities of thiamine and niacin, but not of riboflavin, were lost from the meat to the pickle.   A slight over-all loss of thiamine occurred during this experiment.   The losses of thiamine and of niacin increased considerably as the volume of pickle relative to the meat increased and the loss of riboflavin increased slightly but not significantly.

Effect of Rebuilding Pickles

In commercial practice, spent Wiltshire pickles are usually rebuilt and reused and an attempt was therefore made to assess the effect of this practice on the vitamin losses.   The vitamin contents of a series of commercial pickles

TABLE IV

DISTRIBUTION OF THIAMINE, RIBOFLAVIN, AND NIACIN IN PORK AND PICKLE AFTER CURE AT
VARIOUS MEAT : PICKLE RATIOS

(Averages for three hogs, μgm. per gm. of meat)

Meat : pickle ratio	Pickle	Meat	Total
Thiamine			
Fresh meat	—	11.2	11 2
1.0 : 0.5	2.7	8.1	10.8
1.0 : 0.8	2.8	7.3	10.1
1.0 : 1.0	3.2	7.4	10.6
1.0 : 1.3	3.9	5.9	9.8
1.0 : 2.0	4.1	6.0	10.1
Necessary difference[a]	1.03	1.36	0.78
Riboflavin			
Fresh meat	—	1.51	1.51
1.0 : 0.5	0.13	1.44	1.57
1.0 : 0.8	0.16	1.47	1.63
1.0 : 1.0	0.17	1.47	1.64
1.0 : 1.3	0.20	1.35	1.55
1.0 : 2.0	0.23	1.37	1.60
Necessary difference[a]	—	—	—
Niacin			
Fresh meat	—	55.0	55.0
1.0 : 0.5	10.6	35.0	45.6
1.0 : 0.8	14.8	35.2	50.1
1.0 : 1.0	17.6	36.3	53.9
1.0 : 1.3	19.5	34.3	53.8
1.0 : 2.0	19.0	30.1	49.1
Necessary difference[a]	3.25	9.22	—

[a] *Necessary difference for significance, 5% point.*

increased only slightly during reuse (for three consecutive batches, thiamine 1.1, 1.1, 1.2; riboflavin 0.05, 0.05, 0.07; niacin 2.8, 3.0, and 3.4 μgm. per gm. of meat). Some of the processes used to clarify and rebuild pickles were applied on a laboratory scale and the results are presented in Table V. These results do not necessarily correspond to those that would be obtained under commercial conditions but they indicate that much of the vitamin should be retained in the rebuilt pickle unless carbon black is used as a decolorizing agent.

Effect of Storage and Cooking

Six fresh (pork) and six cured (bacon) rib-in export backs were obtained from a commercial packing concern. These were cut into two pieces and the pieces were individually wrapped in wax paper, overwrapped with kraft, and stored at 30° F. Duplicate cured and uncured pieces were removed at weekly intervals and cut into slices 3 to 4 mm. in thickness. Alternate slices of the longissimus dorsi muscles, trimmed free of extraneous tissue and fat, were used to constitute the cooked and uncooked samples.

TABLE V

VITAMIN CONTENT OF PICKLE AFTER CLARIFICATION, μGM. PER ML.

Treatment of 500 ml. aliquots of pickle	Thiamine	Riboflavin	Niacin
Control	1.1	0.07	5.4
Slurried with 1.6 gm. 'Filter Cel' and filtered	1.1	0.04	5.2
Slurried with 1.0 gm. 'Norit' and filtered	0.4	0.02	1.3
Heated in boiling water bath 60 min., filtered	1.2ᵃ	Nil	5.7ᵃ

ᵃ *No correction applied for evaporation.*

The slices for the cooked sample were broiled until thoroughly done but not browned (500° F. for three minutes on one side, one minute on the other). Both samples were ground and stored at −40° F. until analyzed.

The moisture content of all samples tended to decrease during storage but the losses did not reach the level of statistical significance. During cooking the average moisture content dropped from 70.9 to 56.0% for the pork and from 71.7 to 61.7% for the bacon.

The riboflavin and niacin contents of the samples did not vary significantly during storage but the thiamine content increased irregularly during the first three weeks of storage and then fell again. Although this rise was statistically significant for both the fresh and dry weight data it was probably the result of random differences in the thiamine content of the backs as purchased.

The average vitamin contents of the meat samples before and after cooking are given, on a dry weight basis, in Table VI, together with the percentages

TABLE VI

THIAMINE, RIBOFLAVIN, AND NIACIN CONTENT OF UNCOOKED AND COOKED PORK AND BACON AND THE PERCENTAGE OF EACH VITAMIN RETAINED IN THE COOKED MEAT

(Averages for all storage times, dry weight basis)

	Bacon	Pork	Difference
Thiamine			
Uncooked, μgm. per gm.	56.7	51.3	+ 5.4
Cooked, μgm. per gm.	55.0	42.6	+12.4**
Retention, %	97.3	83.2	+14.1**
Riboflavin			
Uncooked, μgm. per gm.	5.85	6.60	− 0.75*
Cooked, μgm. per gm.	5.64	4.86	+ 0.78*
Retention, %	97.6	73.7	+23.9**
Niacin			
Uncooked, μgm. per gm.	193.1	269.7	−76.6*
Cooked, μgm. per gm.	174.9	228.7	−53.8*
Retention, %	91.3	88.3	+ 3.2

* *Significant to the 5% level.*
** *Significant to the 1% level.*

retained in the cooked samples. The retention of each of the three vitamins in the cooked samples was not significantly affected by storage, but, except for niacin, was greater in the bacon than in the pork.

Discussion and Conclusions

The data obtained during these experiments indicate that little or no destruction of the vitamins occurs during the curing of bacon. With the exception of thiamine in one experiment, all of the vitamin of the fresh meat was present in the meat and pickle at the end of the experiment. Vitamin losses from the meat are, therefore, due to leaching into the pickle. The extent of this leaching is influenced by the amount of exposed lean surface and the volume of pickle, but not, except with riboflavin, by the pickle concentration. The significant effect of pickle concentration on the riboflavin content of the spent pickle can not readily be explained.

Throughout the curing experiments no attempt was made to duplicate commercial practices and the figures for vitamin losses are not to be taken as representative of those incurred in packing plants. Gorham (2) has reported losses of 21.5% for thiamine, 2.7% for riboflavin, and 30.8% for niacin when half-backs were cured on a semicommercial scale using fresh pickle, and Jackson (4) reported average losses of 25.9% of the thiamine, 11.0% of the riboflavin, and 19.2% of the niacin during "wet cure" of bacon on a similar semicommercial basis. These figures do not differ markedly from those obtained in the present work using small pieces of lean meat (24.2% of the thiamine, 11.8% of the riboflavin, and 29% of the niacin). The additional data presented on the effect of the relative amount of pickle, and of the area of lean meat exposed to the pickle, indicate that these results are all high, and that commercial use of a maximum meat : pickle ratio, the curing of whole sides, and the use of rebuilt pickles probably reduce the losses materially.

The results of the cooking experiment are not in complete agreement with those of Gorham (2). The data show that the losses of thiamine and of riboflavin caused by cooking are significantly lower from bacon than from pork, thus confirming a trend suggested by the data of Gorham. But, on the other hand, no significant differences in the percentage of niacin retained in the cooked samples were found in the present work. The samples were cooked for a shorter time (four minutes) than that used by Gorham (six minutes) and the moisture content of the cooked samples was considerably higher (Table VII). With the exception of riboflavin in pork, the losses incurred during cooking were therefore not as great. The losses from bacon were particularly small and any effects of storage were not significant.

The data in Table VII show that the moisture content of the cooked samples was more variable than that of the uncooked samples but the variation is not excessive and the degree of cooking appears to have been satisfactorily controlled. It is interesting to note that there is a significant difference between the moisture content of pork and bacon after cooking for four

TABLE VII

MOISTURE CONTENT OF PORK AND BACON BEFORE AND AFTER COOKING

	Source of data			
	Gorham[a] (averages of duplicates)		Present work (single determinations)	
	No. of samples	Moisture, %	No. of samples	Moisture, %
Uncooked pork	4	73.5 ± 0.79	12	70.9 ± 2.45
Cooked pork	4	50.5 ± 1.50	12	56.0 ± 3 37
Uncooked bacon	10[b]	69.9 ± 1.71	12	71.7 ± 1.62
Cooked bacon	10[b]	48.7 ± 3.98	12	61.7 ± 3.02

[a] *Private communication.*

[b] *Data for 10 samples cured to contain a salt and nitrite content similar to that obtained commercially.*

minutes but not after cooking for six minutes. This difference between the data for the two cooking times makes it appear unlikely that the improved vitamin retentions in the cured samples that were cooked for four minutes are dependent upon the higher moisture retention. It seems probable that the Wiltshire curing process effects a certain degree of stabilization of thiamine, riboflavin, and niacin but the extent of the losses of these vitamins during cooking is influenced by the conditions used and pork and bacon do not necessarily respond equally to a change in the cooking procedure.

Rice *et al.* (7) have shown that the losses during the home cooking of cured ham were lower than those during the cooking of fresh hams. They related this difference to the less severe cooking conditions required with the cured meat. Our samples were cooked under rigidly controlled conditions and the differences found are entirely the result of changes effected in the meat by the curing process. It is conceivable that the differences observed by Rice and his coworkers may also have been due, in part, to a similar effect of the curing process.

The pork and bacon samples used by us were from different pigs and the data for the uncooked samples can not be used for estimating the losses of the vitamins during the curing process. They serve, however, to indicate the relative value of the pork and bacon as a source of these vitamins, and it is apparent from the data that the stabilization of thiamine and riboflavin effected by the curing process was sufficient to offset the losses incurred during processing of the bacon. The cooked bacon is thus a better source of these two vitamins than is the cooked pork. Niacin, however, was lost, during cooking under these conditions, to almost the same extent from both the pork and the bacon and the cooked bacon therefore appears to be a poorer source of this vitamin than the cooked pork.

Acknowledgments

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CHEMICAL AND MICROBIOLOGICAL STUDIES ON STORED, SALTED BUTTER[1]

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Abstract

Salted butter from two Canadian plants was stored in parchment wrappers and in cans at −1.1°, 10.1°, 21.1°, and 32 2° C. (30°, 50°, 70°, and 90° F.). Deterioration of the stored samples was followed by assessment of flavor, and by determination of: total acidity, free acidity, amino acid, pH, and fluorescence of the butter serum; peroxide oxygen, free fatty acids, and fluorescence of the butterfat; acidity of the whole butter; the number of viable, proteolytic, lipolytic, and oxidase positive organisms; and yeast and mold counts.

Butter in cans deteriorated less rapidly than print samples although differences were less pronounced at 70° and 90° F. than at 30° and 50° F. Canned samples did not change appreciably in quality during 45 weeks' storage at 30° F. In general, decomposition of the serum and flavor deterioration became evident before changes occurred in the fat fraction. Stability of the serum was the limiting factor in keeping quality. The formation of amino acids and intermediate products of protein decomposition contributed largely to flavor changes.

Flavor scores were more closely related to chemical objective test values than to microbiological counts. Storage temperature showed a marked influence on the correlation of objective test values with flavor score. Of the objective tests studied, total serum acidity (amino acidity plus free serum acidity) pH and serum fluorescence were most closely associated with flavor score.

Introduction

The production of butter is seasonal and is usually concentrated in areas where fluid milk is not readily marketed. These factors emphasize the importance of keeping quality if butter is to reach the consumer in good condition. Many studies have been made to improve methods of handling the cream and of packaging butter. It has been reported that butter develops tainted flavors when stored in wooden containers (8, 11, 18). Partial success in preventing the production of these flavors by the use of various types of liners has been reported (2, 3, 17).

Butter has been shipped to countries in the tropics for many years, often arriving in poor condition, and consumers there have become conditioned to using a product that is undesirable by temperate zone standards. This state of affairs is accepted as inevitable by consumers and exporters alike, and no particular effort has been made to improve the quality of canned butter for export. During the war, canned butter was included in Red Cross parcels and in special service rations. In an effort to extend the storage life of butter for these purposes, greater attention than heretofore was given to initial quality.

The information available about keeping quality is contradictory and relates chiefly to studies of accelerated shelf tests. This investigation was carried out to obtain factual data on changes in butter at storage temperatures above those used in warehouses (i.e. −10° to 0° F.). As butter contains more than one component, it is logical that storage temperature may affect one component more than another and thus deterioration may be favored in specific fractions at different temperatures. Therefore, the butter was stored over a range of temperatures to try to establish the relative susceptibility of different components at high and low temperatures.

Many factors have been suggested as responsible for spoilage in butter, and it was felt that no one test would satisfactorily assess keeping quality. Although organoleptic tests measure the over-all quality of butter, they lack uniformity between investigators. Therefore, a number of chemical and microbiological determinations were carried out on whole butter, butter serum, and butterfat from stored whole butter.

The present study compares the keeping quality of canned and print butter over a wide range of temperatures using a number of chemical and microbiological methods. The degree of association between the results of these methods and organoleptic assessment was also determined.

Experimental

The experimental material consisted of two lots of commercial first grade Canadian salted butter prepared from pasteurized cream. One lot was selected from a single churning of Western butter and the second was prepared from selected neutralized cream in an Eastern creamery. Part of the latter lot was canned immediately in lacquered cans without parchment wrapping and an equal part prepared in parchment-wrapped 2-lb. prints. The Western butter churning was selected on the basis of low yeast and mold counts and an accelerated storage test. Cylindrical 1-lb. prints were then prepared and wrapped in parchment; half of these were sealed in lacquered cans. All the material was shipped by refrigerated car to Ottawa and held at −40° F. until allotted at random to experimental conditions.

The canned and the print butter from both plants were stored at 30°, 50°, 70°, and 90° F. The prints were stored in sterile beakers covered with brown wrapping paper. Samples were withdrawn at intervals depending on storage temperature: at 30° F., after 16, 32, 64, 128, 192, 256, 321, and 385 days; at 50° F., after 4, 8, 16, 32, 64, 96, 128, 192, and 256 days; at 70° F., after ½, 1, 2, 3, 4, 8, 16, 32, 64, and 128 days; and at 90° F., after ¼, ½, 1, 2, 3, 4, 8, 16, and 32 days.

Methods

A quantitative determination by chemical methods of the substances causing off-flavors in butter is difficult, because of the complexity of the reactions accompanying spoilage. However, chemical and microbiological methods are undoubtedly of value for comparison of similarly treated products.

The following objective measurements were selected for study: peroxide oxygen, free fatty acid, Kreis value, and fluorescence of the fat fraction; acidity of whole butter; amino acid by Sorensen's titration, and acidity and fluorescence of the butter serum. Viable, lipolytic, proteolytic, and oxidase positive organisms; and yeast and mold counts on the whole butter were also included.

Preparation of the Sample for Analysis

Samples for microbiological examination were taken first with sterile cork borers, made of stainless steel. The butter stored at 90° F. was very soft, necessitating cooling for a few hours at 45° F. When time did not permit hardening by cooling, the wrapper was removed, the sample mixed, and representative portions taken with a sterile tongue depressor. The square prints were sampled through the face opposite to that on which the parchment flaps met. With the round prints, borings were taken through the face just past the paper overlap. The cans were flamed on top and opened with a wheel-type can opener that had been flamed. The lid and cover paper were removed and borings taken at and around the center. Enough material was taken from the cans and prints to give at least 20-ml. portions when melted. Samples of whole butter were withdrawn for immediate organoleptic rating and whole butter acidity measurements.

Serum and fat were separated by a method previously described (10). Briefly, the butter is heated in centrifuge bottles placed on a steam bath at 212° F., stirred (with a thermometer) until it reaches 110° to 120° F., then centrifuged for 10 min. at 1700 r.p.m. The fat is removed by siphoning and filtering through absorbent cotton, and part of the clear portion of the serum is pipetted into a separate container.

Organoleptic Ratings

Two sets of organoleptic ratings were obtained. The butter was scored for flavor only by three professional graders, by methods outlined in the Canadian Dairy Industry Act and Regulations, 1942, pp. 48 to 51. The second system, which permitted statistical computations, employed a panel of 10 judges to assess flavor as follows: 10, excellent; 8, good; 6, fair; 4, poor; 2, bad; 0, unapproachable. At any one time the 10 tasters were required to score a set of four samples chilled to approximately 50° F.

Acids

The free fatty acids of the butter fat were determined by titrating 10 gm. dissolved in 50 ml. of hot ethanol with $0.02N$ sodium hydroxide, until neutral to phenolphthalein. This procedure was also used to determine acidity of the whole butter, which includes other titratable acids formed from protein and carbohydrate breakdown. A colorimetric method (13) was tried but proved to be inadequate.

A difference in amino nitrogen, as determined by Van Slyke's method (19, 20), was obtained between serums of fresh and spoiled butter, (0.073 mgm. of amino nitrogen per ml. of serum for fresh butter, 0.159 mgm. for

spoiled butter). This finding with the evidence of other investigators (15) suggested that protein deterioration was taking place. The acidity of the serum due to free acids, such as lactic, was determined by titrating an aliquot with 0.02 N sodium hydroxide, employing phenolphthalein as an indicator. The values are reported as mgm. of sodium hydroxide per 100 ml. of serum, and are termed free serum acidity. To obtain an estimate of the protein decomposition, the formation of amino acids was followed by Sorensen's titration. Although this method has its limitations, it enabled comparison between samples. The values are reported as mgm. of sodium hydroxide per 100 ml. of serum and are termed amino acids.

To estimate the over-all change in the serum, the values for free serum acidity and amino acids were combined and termed total serum acidity.

The hydrogen ion concentration of the serum was determined at 77° F. on 10 ml. of the serum by means of a pH meter.

Fluorescence

Since many types of fat show changes in fluorescence with storage (16), it was considered desirable to examine the change in the fat from stored butter. One-gram samples of butterfat from fresh and spoiled butter were dissolved in 10 ml. portions of solvents and their fluorescence measured on a Coleman photofluorometer with filters transmitting light in the region of 365 mμ. The fluorescence readings are given in Table 1. Of the solvents used, benzene

TABLE I

FLUORESCENCE OF BUTTERFAT DISSOLVED IN ORGANIC SOLVENTS

Solvent	Fresh butter	Spoiled butter	Difference
Benzene	64.5	41 0	23 5
Xylene	58.5	37.0	17.5
Dioxane	51 5	38 0	13 5
Ethyl ether	30 0	22.5	7.5
Ethylene dichloride	37.5	31 0	6 5
Amyl acetate	32.0	26 0	6.0
Petroleum ether	39 5	34 0	5.5
Acetone	15.5	17 5	2 0
Chloroform	21.5	20 0	1 5
Carbon tetrachloride	6.5	8.0	1 5
Ethanol	Too turbid	—	—
Methanol	Too turbid	—	—

and xylene resulted in the largest difference between fresh and rancid fat. Xylene was selected for further study. The effect of fat concentration was also investigated. The results in Table II indicate that the fluorescence of butterfat from fresh and spoiled butter increased with fat concentration, and also that the differences between fresh and spoiled butter were larger at the higher concentrations. As the differences between the spoiled and fresh butter were not the same at all concentrations tested, a standard concentration of

10% butter fat was chosen.   However, as the test was not totally satisfactory, it was applied only to a limited number of samples.   Serum fluorescence was determined as described previously (10).

TABLE II

THE EFFECT OF BUTTERFAT CONCENTRATION IN XYLENE ON FLUORES-
CENCE VALUES

Percentage of butterfat	Fresh	Spoiled	Difference
10	62.0	45.0	17.0
20	85.5	64 0	21.5
30	96.0	74.0	22.0

Peroxide Oxygen

Both the ferrometric and iodometric methods (5, 7) were employed for the estimation of peroxide oxygen value, for reasons outlined in a recent investigation on lard (9).

Kreis test

The modification by Walters *et al.* (21) of the original Kreis test (14) was selected as the most suitable because of its simplicity and development of color in a single phase system.   Investigation by White (22) showed that the color intensity was proportional to the concentration of fat.   One gram of butter oil was employed in each test.   If the intensity of color after the reaction was too great, a dilution with solvent was made.   The Evelyn photoelectric colorimeter (6) provided with a 580 Rubicon filter was found to be quite satisfactory for measurement of color intensity.   The results are reported as modified extinction coefficients.   Although the values obtained have little theoretical significance, they are quite suitable for comparison between samples.

Microbiological Methods

Samples were held at 40° F. until they could be plated.   They were then melted at 110° F. and 10 ml. pipetted into a 90 ml. water blank preheated to 110° F.   Appropriate dilutions were made in the blanks.

Viable counts were made on tryptone glucose agar with 0.5% skim milk added (1, p. 22).   Both proteolytic (caseinolytic) and viable organisms were estimated on these plates, counts being made at two and three days after incubation at 90° F. and three and five days at 70° F.   Yeasts and molds were estimated on Difco potato dextrose agar adjusted to pH 3.5 to 4.0 just before use.   Counts were made after three and five days at 70° F.   For the estimation of lipolytic organisms, butterfat was prepared from butter having a low free fatty acid content and no iodometric peroxide oxygen value and stained with neutral red base (12).   Five per cent of stained fat was added to 2% agar containing 0.5% each of Difco proteose–peptone and tryptone.

Only colonies imparting a distinct red color to the fat globules under or around them were considered lipolytic. Oxidase positive organisms were detected on the same medium by using unstained fat and flooding the plates with a weak solution of dimethylparaphenylenediamine. Both lipolytic and oxidase positive organisms were counted after five days at 90° F. and seven days at 70° F.

Treatment of Data

The many data required statistical analyses to assess the relative importance of the factors involved. Each test is reported first from the standpoint of treatment effects. These effects are shown in detail in graphical form in Figs. 1 to 4, in which the results from the two plants are averaged, as it was more desirable to emphasize differences due to temperature and packaging than those due to source. The relation of chemical tests to flavor, and their assessment of keeping quality, are indicated by correlation and regression coefficients and an analysis of covariance (Tables IV to VI).

Results

Flavor

The mean flavor scores as assessed by the laboratory flavor panel are shown in Fig. 1. An increase in storage temperature or time caused a decrease in flavor score. The flavor score of the print butter decreased more rapidly than did that of canned butter at all the storage temperatures. There was only a slight decrease in flavor score of the canned butter after storage for 45 weeks at 30° F. At 50° F., the canned butter decreased in the first 14 weeks, then showed a definite improvement.

Further details of flavor results obtained by the butter graders and laboratory flavor panel are given in Appendix Tables I and II. For the purpose of this investigation the laboratory flavor assessment system was superior to that employed commercially as it could be correlated more easily with objective tests and furnished a more reliable assessment of the butter flavor by employing a larger group of tasters and a scoring system with equal increments.

Fluorescence

An increase in storage time or storage temperature resulted in an increased serum fluorescence value (Fig. 1). The rate of increase in the fluorescence was greater at 70° and 90° F. than at 30° and 50° F. The print butter showed a greater increase in serum fluorescence values at all the storage temperatures than that stored in cans. The canned butter stored at 30° and 50° F. showed approximately six units increase in serum fluorescence in 45 weeks.

As the fluorescence determinations on the fat fraction were not very informative, only a few examples are given in Table III. The fluorescence of the fat fraction did not change appreciably with flavor deterioration at any of the storage temperatures.
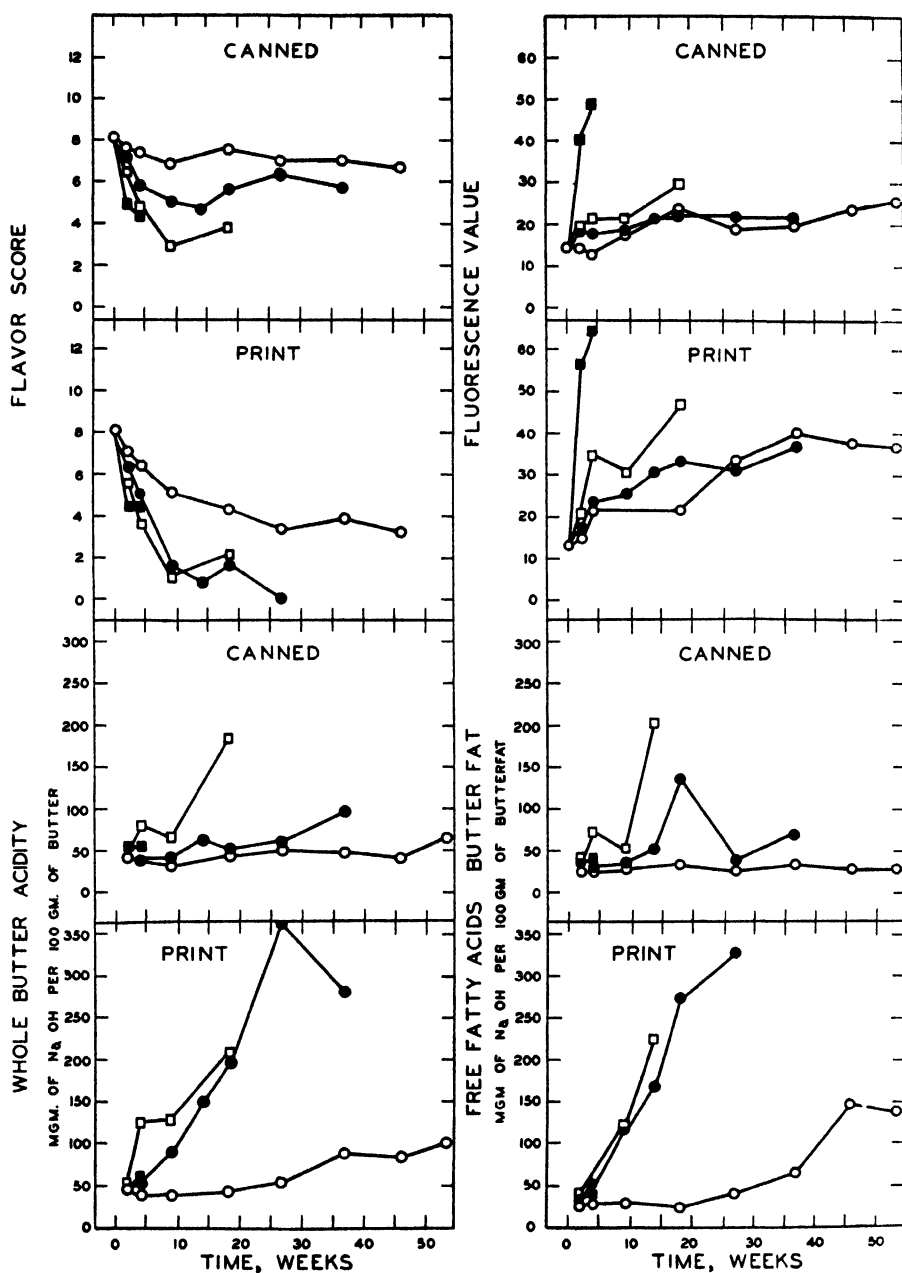
FIG. 1. *Effects of storage at 30°, 50°, 70°, and 90° F. on the laboratory flavor panel score, serum fluorescence, whole butter acidity, and the free fatty acid content of butterfat from canned and print, salted butter.   30° F., O; 50° F., ●; 70° F., □; 90° F., ■.*

TABLE III

FLAVOR SCORES AND CHEMICAL MEASUREMENTS ON THE FAT FRACTION FROM BUTTER STORED
AT 30°, 50°, 70°, AND 90° F.

(Values averaged over both plants and both packages)

Storage Temperature, °F.	Flavor score	Peroxide oxygen content		Kreis value	Fluorescence value
		Ferrometric	Iodometric		
90	8.1	0.0	0	0.3	60
	4.7	3.8	0	0.6	57
	4.4	1.7	0	0.7	56
70	8.1	0.0	0	0.3	60
	6 0	3.7	0	0.5	55
	4.2	1.5	0	0 6	57
	2.0	2.0	0	2 0	56
50	8 1	0.0	0	0.3	60
	6.8	2.8	0	0 4	56
	5.4	1.3	0	0.5	58
	3 3	1.6	0	0 3	56
	3 2	2.2	0 3	8 2	58
	2.8	5.1	1.3	6 5	—
30	8 1	0.0	0	0.3	60
	7.3	2 7	0	0 4	—
	6 8	1.8	0	0 4	55
	7.5	1.4	0	—	58
	7.0	2 5	0	—	56
	6.8	1.6	0	4 0	—
	5 8	—	0	- ·	—

Acids

The acidity determinations on the whole butter generally showed an increase with storage time and storage temperature for both canned and print wrapped butter (Fig. 1). The increase was more pronounced for print than for canned butter. The formation of acidity was suppressed in the canned butter stored at 30° and 50° F., and as this measurement is some indication of the hydrolysis of protein and fat, it is inferred that canning the material retarded the rate of spoilage. The canned butter showed only a slight increase in acidity after storage for 45 weeks at 30° F.

The free fatty acid content of the butter fat increased with storage time and storage temperature for both canned and print material (Fig. 1). There was a more prominent increase in the free fatty acids in the butter stored in prints than that stored in cans. There was little change in free fatty acids in canned material even after storage at 30° F. for 45 weeks.

From Fig. 2 it may be seen that the free serum acidity increased with storage time, the rate varying with the storage temperature. The butter stored in prints showed a greater rate of increase at all the storage temperatures. The butter stored at 30° F. in cans showed very little increase after 45 weeks, while that stored in the prints showed a substantial increase after 20 weeks.
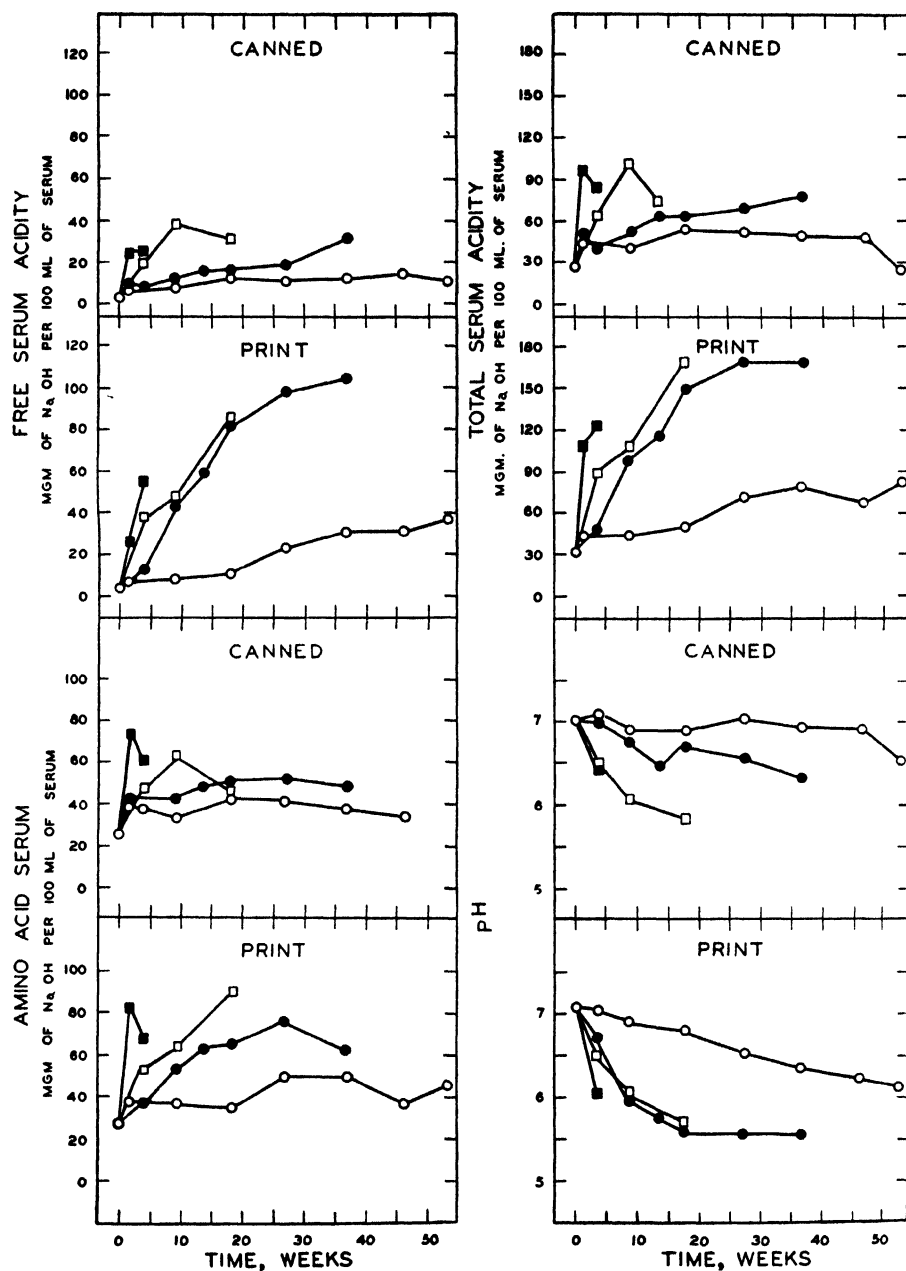
FIG. 2. Effects of storage at 30°, 50°, 70°, and 90° F. on free serum acidity, amino acid in the serum, total serum acidity, and pH of the serum from canned and print, salted butter. 30° F., ○; 50° F., ●; 70° F., □; 90° F., ■.

Canning of the butter appeared to suppress the formation of free serum acidity most at 30° and 50° F.

The amino acids showed an increase followed by a decrease with storage time (Fig. 2). This was accentuated at the higher storage temperatures. The rate of increase in the amino acids was greater at 70° and 90° F. than at 30° and 50° F. At all the storage temperatures the amino acid content developed somewhat more rapidly in the print butter than in the canned butter. The canned material stored at 30° F. showed little change after the first two weeks.

Increases in total serum acidity with period of treatment for both canned and print butter at all the storage temperatures may be seen from Fig. 2. In some cases, there was first an increase, then a decrease after prolonged storage. This was quite evident in butter stored in cans at 70° and 90° F. At all storage temperatures, print butter showed larger increases in total serum acidity than canned butter. From this evidence, it is apparent that the over-all changes in the serum were greater for material stored in prints.

The hydrogen ion concentration of the butter serum decreased with storage time (Fig. 2), the decrease being more rapid at the higher storage temperatures. The changes in print butter were greater than those in canned material. As the pH is a function of the fermentation taking place, it is considered that very little deterioration from this source took place in the canned butter stored at 30° F. for 45 weeks.

Peroxide Oxygen Content, Kreis Value

The results of determinations on the fat fraction are shown in Table III. The peroxide content as measured by both methods showed no large increase with flavor deterioration at any of the storage temperatures. The aldehyde content as measured by Kreis value showed a slight increase with flavor deterioration at the lower temperatures. The lack of appreciable increases in peroxide oxygen content and aldehyde with flavor deterioration suggests that oxidative changes in the fat fraction contribute little to the flavor deterioration in stored salted butter.

Microbiological Tests

Since there was little difference in the counts after incubation at 70° and 90° F., averages were taken, and since differences between conditions of storage were of primary interest, the results for butter from both sources were also averaged for graphical presentation. Owing to sampling errors, the bacteriological estimations show considerable variation. However, the data presented in Figs. 3 and 4 show the trends found at various storage temperatures.

The original number of both the viable and proteolytic organisms was slightly greater in the canned than in the print butter. In the canned material, there was a small but rapid increase during the first two days at 70° and 90° F. The general trends with all types of organisms were very similar in butter
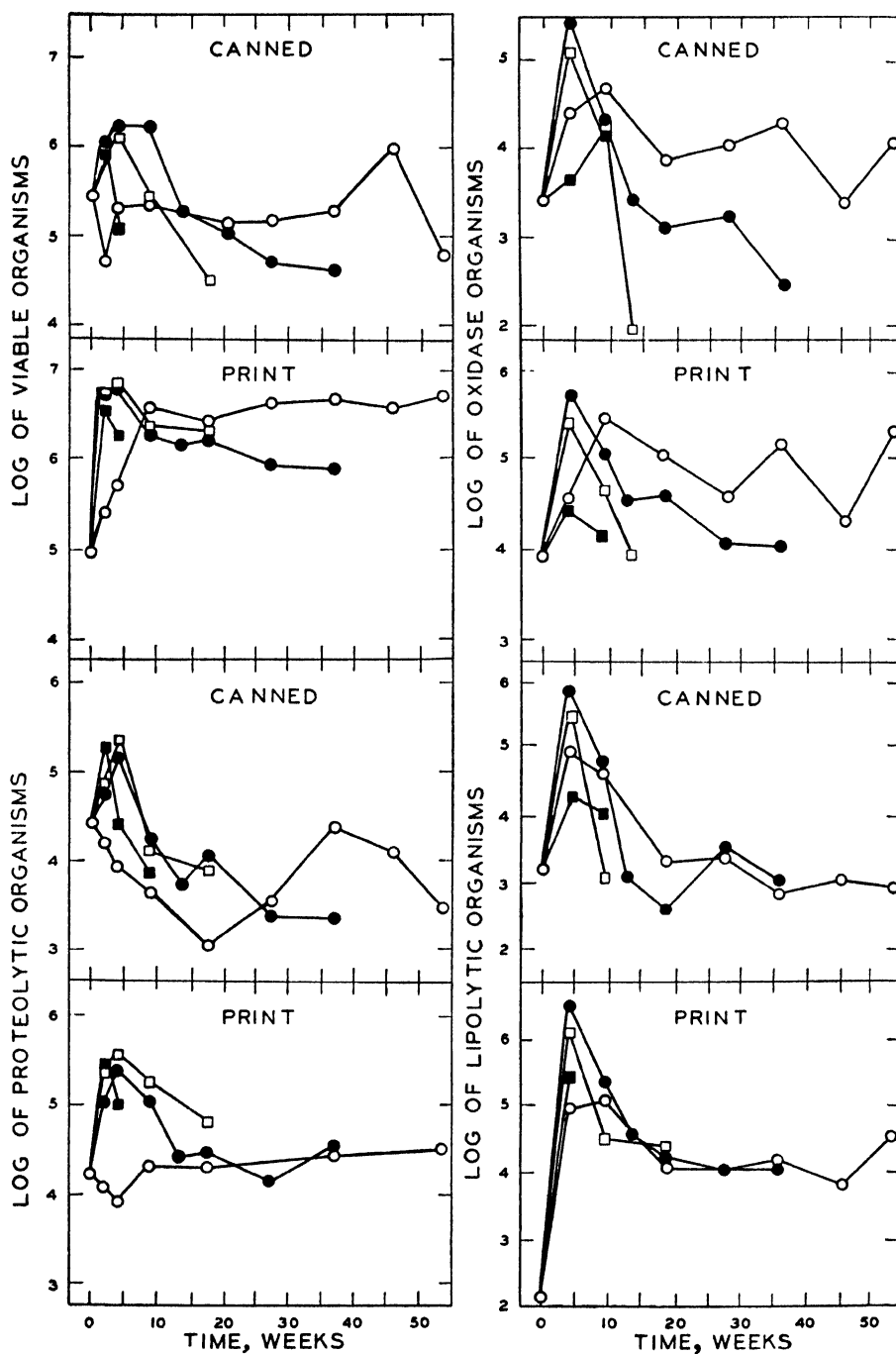
FIG. 3. *Effects of storage at 30°, 50°, 70°, and 90°, F. on viable, proteolytic, lipolytic, and oxidase positive organism contents of canned and print, salted butter.   30° F., O; 50° F., ●; 70° F., □; 90° F., ■.*

stored at 90° F.   The number of bacteria reached a peak in three to four days, remained at approximately that level for 8 to 16 days, and then decreased. At 70° F., the peak came in four to eight days and the numbers remained fairly constant for 32 days.   At 50° F. the increase was slower, the numbers
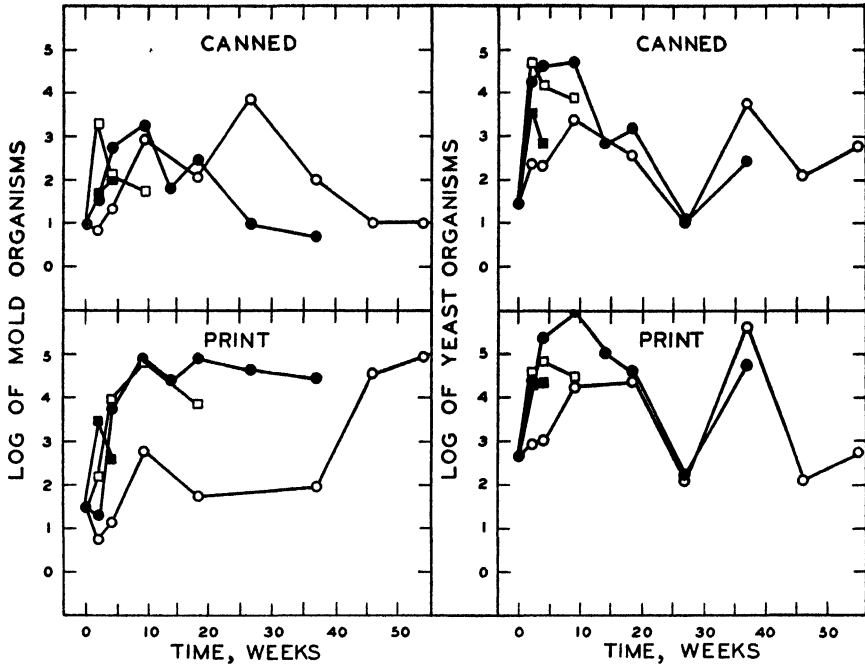

Fig. 4.   *Effects of storage at 30°, 50°, 70°, and 90°, F. on mold and yeast organism contents of canned and print, salted butter.   30° F., O; 50° F., ●; 70° F., □; 90° F., ■.*

reaching a maximum in 16 to 32 days, and then decreasing after approximately 64 days.   At 30° F., the trends were more uniform and the peaks were reached in from 32 to 70 days and in some cases even longer.   In general, the changes in the canned butter were not so great as in the print and the organisms did not survive so long.   In the prints, the number of organisms usually remained at a level as great as or greater than the original number, whereas in the canned butter the levels were usually below the original values.

The numbers of total viable organisms (Fig. 3) at both 70° and 90° F. rose rapidly, the increase in the prints being about 10 times greater than in the canned.   In the butter stored at 50° F., the increase was more rapid in the prints and greater numbers were attained.   At 30° F., there was little increase in the canned material, but in the prints the increase was decided and the number of organisms remained at a high level throughout the experiment.

The number of proteolytic organisms showed trends similar to those of the viable count (Fig. 3).   At 30° F., there was very little increase.   The decreases after the initial increases were somewhat steeper than for the total count.

The oxidase producing organisms increased at a much slower rate in the canned butter than in the prints (Fig. 3). However, in the canned material the numbers eventually decreased at the higher temperatures to levels equal to or lower than the original number. For the prints the decrease was slower, but counts finally reached the original level, except in the prints stored at 30° F.

Lipolytic organisms increased from a low original content of about 140 per gm. to about a million per gram in the print butter stored at 50° and 70° F. (Fig. 3). At the three lower storage temperatures, the average number remained at above 10,000 per gram for 20 weeks. In the cans the increase, although not great, was maintained over a similar period, and the number then dropped to approximately the original value.

The yeast count showed a greater increase in the canned butter than in the prints (Fig. 4). For both the print and canned butter stored at 30° F., there was a general increase for at least the first 35 weeks. The drop shown at the 27th week is due to a poor lot of media. Development of molds was much greater in the prints than in the canned material. This is readily seen from Fig. 4. The counts remained high in the prints, but in the canned butter there was some reduction after the first few weeks. Darkening of the surface due to molds was first noticed in the prints in 32 days at 70° F., in 64 days at 50° F., and in 192 days at 30° F. Light mold growth was noted a few times on the surface of the canned butter but never became distributed throughout the mass as in the prints.

Relation Between Flavor and Objective Test Values

The relation between flavor as assessed by the laboratory flavor panel and objective test values for each storage temperature was assessed by computing correlation and regression coefficients. An objective test, to be of value for general assessment of keeping quality, should have a correlation coefficient of at least .8 and there should be no significant difference in regression values between sets of data from material stored under different conditions. The significance of any difference in regression values for the butter stored at the four temperatures was determined by analysis of covariance.

The correlation coefficients of the relations between flavor and the objective tests are given in Table IV. All the objective test values increased with a decrease in the flavor score, except pH, which decreased. The chemical objective tests were more closely related to flavor changes than the bacteriological tests. The correlation coefficients between flavor and viable, proteolytic, oxidase, and yeast counts attained significance for storage at 30° and 90° F. while those for 50° and 70° F. were not significant. Lipolytic count correlated significantly with flavor only for 90° F. storage. Of all the bacteriological tests studied, the mold count gave the highest correlation coefficient with flavor and attained statistical significance for all temperatures except 30° F. Although the correlation coefficient between flavor and mold count was much higher than for the other bacteriological tests, they were still too

TABLE IV

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN FLAVOR SCORES AND OBJECTIVE TEST VALUES FOR SALTED BUTTER STORED AT 30°, 50°, 70°, AND 90° F.

Quantities correlated:	30° F.		50° F.		70° F.		90° F.	
Flavor score with:	Degrees of freedom	r	Degrees of freedom	r	Degrees of freedom	r	Degrees of freedom	r
Viable count	29	−.56**	35	−.14	39	−.07	39	−.33*
Proteolytic count	28	−.36*	35	−.01	39	−.07	39	−.44**
Lipolytic count	29	−.27	35	−.10	38	−.12	39	−.46**
Oxidase positive count	29	−.45**	35	.01	39	−.23	39	−.34*
Yeast	29	−.59**	35	−.24	37	−.01	39	−.37*
Mold	29	−.30	35	−.84**	38	−.65**	39	−.36*
Butter acidity	30	−.59**	38	−.71**	41	−.79**	36	−.67**
Free fatty acids	30	−.61**	38	−.82**	41	−.71**	36	−.21
pH	30	.63**	38	.84**	41	.82**	36	.51**
Fluorescence	30	−.63*	38	−.69**	40	−.75**	36	−.84**
Amino acid	20	−.28	26	−.50**	22	−.61**	20	−.75**
Free serum acidity	20	−.75**	26	−.92**	22	−.67**	20	−.71**
Total serum acidity	20	−.82**	24	−.72**	26	−.86**	20	−.64**

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

low to predict flavor score with any degree of reliability. The whole butter acidity, free fatty acids, and pH showed higher correlation with flavor for the material stored at 50° and 70° F. than for that stored at 30° and 90° F. Correlation coefficients between flavor and serum fluorescence and amino acids increased with an increase in storage temperature. Of the chemical tests studied, free serum acidity and total serum acidity gave the highest correlation coefficients with flavor.

The regression coefficients between flavor and serum fluorescence, whole butter acidity, free fatty acids, free serum acidity, amino nitrogen, total serum

TABLE V

REGRESSION COEFFICIENTS BETWEEN FLAVOR SCORES AND OBJECTIVE TEST VALUES FOR SALTED BUTTER STORED AT 90°, 70°, 50°, AND 30° F.

Quantities correlated: Flavor score with:	90° F.		70° F.		50° F.		30° F.	
	Degrees of freedom	β	Degrees of freedom	β	Degrees of freedom	β	Degrees of freedom	β
Free fatty acids (whole butter)	36	− .087	41	−.027	38	−.017	30	−.058
Free fatty acids (butter fat)	36	− .019	41	−.021	38	−.016	30	−.054
pH on serum	36	1.35	41	2.94	38	3.49	30	2.70
Fluorescence value on serum	36	− .064	40	−.160	38	−.207	30	−.100
Amino nitrogen on serum	20	− .066	22	−.104	26	−.074	20	−.046
Free serum acidity value	20	− .060	22	−.073	26	−.061	20	−.132
Total serum acidity value	20	− .040	22	−.052	26	−.043	20	−.069

acidity, and pH on the serum for each storage temperature are given in Table V. It is quite evident that a difference in regression coefficients exists between the storage temperatures and the regression coefficients for pH are greater than for the other measurements. The significance of these differences was assessed by analysis of covariance (Table VI). No significant

TABLE VI

ANALYSIS OF COVARIANCE WITH STORAGE TEMPERATURES OF REGRESSION COEFFICIENTS BETWEEN OBJECTIVE TESTS AND FLAVOR SCORE

Tests with flavor	Average regression coefficient		Individual coefficient		Difference in regression coefficients		Residual (pooled)	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Fluorescence	1	235.42**	4	72.99**	3	18.85**	146	1.96
Free fatty acid (whole butter)	1	228.50**	4	67.23**	3	13.48**	138	1.90
Free fatty acid (butterfat)	1	242.36**	4	69.99**	3	8 53**	138	1 89
Free serum acidity	1	265.95**	4	69.78**	3	4 39	94	2 00
Amino nitrogen	1	127.19**	4	33.81**	3	2.68	94	3.65
Total serum acidity	1	263.60**	4	67.53**	3	2 16	94	2 18
pH on serum	1	334.39**	4	119.42**	3	47.77**	154	0 73

** *Indicates 1% level of statistical significance.*

difference in regression coefficients between storage temperatures was found for the relations between flavor score and free serum acidity, amino nitrogen, and total serum acidity. Significant differences between the storage temperatures were evident for the regression coefficients between flavor and whole butter acidity, free fatty acids, pH, and serum fluorescence.

Interrelation of the Chemical Measurements

The interrelation of the chemical measurements was assessed by computing correlation coefficients. The results are shown in Table VII. The fluorescence values were most highly associated over all the storage temperatures with amino acids and total serum acidity, and least with pH. From these results, it may be inferred that fluorescent materials are derived from amino acids or a reaction in which amino acids are involved. Free serum acidity and amino acids were not so closely associated as the amino acids and total serum acidity. This indicates that free serum acidity may be produced by microbiological action without significant production of amino acids. The pH was significantly correlated with all measurements but least so with amino acid content. A storage temperature of 30° F. gave a much lower association between the free fatty acids and other measurements except whole butter acidity. The acids content of the whole butter and butterfat were significantly correlated with the other measurements.

TABLE VII

SIMPLE CORRELATION COEFFICIENTS FOR THE INTERRELATION BETWEEN OBJECTIVE TESTS ON BUTTER STORED AT 30°, 50°, 70°, AND 90° F.

	Storage temp., °F.	Correlation coefficients					
		Butter acidity	Free fatty acids	pH	Amino acids	Free serum acidity	Total serum acidity
Fluorescence	90	.65**	.76**	−.21	.85**	.79**	.92**
	70	.82**	.90**	−.48**	.86**	.71**	.83**
	50	.70**	.77**	−.50**	.84**	.64**	.77**
	30	.70**	.72**	−.26	.67**	.59**	.79**
Total serum acidity	90	.87**	.93**	−.85**	.89**	.89**	
	70	.77**	.78**	−.76**	.88**	.95**	
	50	.82**	.90**	−.77**	.80**	.83**	
	30	.42*	.61**	−.27	.80	.77**	
Free serum acidity	90	.87**	.77**	−.84**	.58**		
	70	.75**	.81**	−.80**	.69**		
	50	.40	.91**	−.17	.34		
	30	.53**	.74**	−.74**	.25		
Amino acids	90	.66**	.62**	−.60**			
	70	.55**	.57**	−.54**			
	50	.58**	.63**	−.34			
	30	.23	.20	−.28			
pH	90	−.87**	−.60**				
	70	−.62**	−.92**				
	50	−.70**	−.73**				
	30	−.53**	−.55**				
Free fatty acid	90	.78**					
	70	.96**					
	50	.86**					
	30	.90**					

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

Discussion

Many investigators have employed mold, yeast, and bacterial counts to assess the keeping quality of butter. In the present investigation, temperature had a pronounced effect on the association between microorganisms and flavor. Higher correlations were found between flavor score and the numbers of proteolytic, lipolytic, and oxidase producing organisms at 30° and 90° F. than at 50° and 70° F. This finding, and the fact that considerable difficulty is encountered in estimating the true number of organisms present, make it unlikely that any microbiological test employing present methods would prove to be satisfactory for predicting flavor score.

The measurements on the serum fraction were the most promising of the chemical methods investigated for predicting flavor score during storage. In a previous investigation (10) fluorescence measurements of the serum showed possibilities as a test for estimating keeping quality. The present evidence

confirms its usefulness as a guide for investigational purposes, especially under accelerated storage conditions (70° and 90° F.). However, the flavor tests remain the best method of estimating keeping quality.

The free serum acidity, amino acids, and total serum acidity showed no significant differences in regression coefficients between storage temperature, indicating that the rate of formation of acids with flavor score decrease was the same for all the storage temperatures studied. This is one of the essential requirements of an objective test of flavor deterioration. However, the amino acids showed low correlation coefficients at some of the storage temperatures, which prevents their use as a keeping quality test. The remaining two tests were closely associated with flavor deterioration, but no prediction equations were calculated, as it was deemed desirable to base such equations on data from samples obtained from many sources and stored under varying temperatures and humidities. Moreover, because of the complexity of the reactions involved in butter deterioration, it would seem desirable to use more than one test in constructing prediction equations for assessing keeping quality.

Of the objective tests used on the serum fraction the commonly used pH measurement was useful for assessing flavor deterioration in butter stored at low temperatures but not in butter stored at high temperatures. Free serum acidity, total serum acidity, and fluorescence may be applied under accelerated storage conditions to differentiate between butters of good and poor keeping qualities.

As no appreciable oxidative decomposition of the butterfat could be detected by tests of peroxide oxygen or aldehyde content, the free fatty acids of the butterfat were evidently developed by the action of lipolytic enzymes. This lack of appreciable increase in peroxide oxygen and aldehyde content indicates that oxidative changes in the fat fraction contribute little to flavor deterioration in stored salted butter. However, the free fatty acids developed by lipolysis may exert some influence at high storage temperatures.

Decomposition of the serum and flavor deterioration were evident before any free fatty acid or oxidative changes could be detected in the fat. As shown by other workers (4), the antioxidant effects of decomposition products of the serum, e.g., amino acids, may account for the stability of the fat fraction. Flavor deterioration is evidently due to protein decomposition rather than to changes in the fat fraction. The decrease in association between flavor score and amino acids with a decrease in storage temperature indicates that the serum protein was not so completely decomposed at the lower temperatures, that intermediate products are responsible for flavor deterioration, and that deterioration reactions are not the same at all temperatures.

From the results of the chemical and microbiological tests it is apparent that deterioration is retarded in canned butter, especially at the lower temperatures studied. Indeed, canned butter can be stored at 30° F. for periods up to 45 weeks without appreciable lowering of its quality. Therefore canned butter can be stored and transported at chill temperatures rather than at the freezing temperatures required for butter in prints.

Acknowledgments

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APPENDIX TABLE I

FLAVOR PANEL SCORES AND OFFICIAL GRADERS' SCORES OF BUTTER FROM PLANT A

Storage temperature, °F.	Storage time, days	Canned		Print	
		Panel	Graders	Panel	Graders
	Initial	7.2	39.0	7.5	38.5
	16	7.7	40.0	7.2	39.5
	32	7.5	38.5	5.9	38.0
	64	6.1	38.0	3.7	39.0
30	128	7.4	39.0+	4.3	39.5
	192	6.6	39.0	4.1	—
	256	7.6	38.0	4.0	36.0−
	321	6.5	39.0	2.8	37.0
	385	6.9	—	0.0	—
	Initial	7.2	39.0+	7.5	38.5
	4	7.3	39.0	7.4	39.0
	8	7.0	39.0	6.9	38.5
	16	7.1	39.0−	6.8	39.5
	32	6.5	36.5	5.6	38.5
50	64	3.3	37.0	0.9	36.0
	96	4.9	36.0	2.4	36.0
	128	4.9	—	0.7	—
	192	6.1	37.5	2.0	—
	256	5.9	38.0	0.0	—
	Initial	7.2	39 0+	7.5	38.5
	0 5	7.3	38.5	7.4	38.5+
	1	7.0	39.0−	6.9	39.0
	2	7.3	39.0−	7.2	38.5
70	3	7.0	39.0	6.7	39.0−
	4	6.8	39.0	6.9	39.0−
	8	6.5	37 5	6.2	39.0+
	16	6.1	38.5	6.1	37.5
	32	5.1	37.0	4.4	36.5
	64	3.2	38.0	1.2	35.0
	128	4.2	—	1.6	—
	Initial	7.2	39.0+	7.5	38.5
	0.25	7.4	39 0+	7.5	39 5
	0 5	—	37.0	—	38.5
	1	6.7	38.5	6.5	39.0
	2	6.0	39.0−	6.2	38 5
90	3	6.3	39.0	6.4	39.0−
	4	6.3	39.0−	6.2	38.5
	8	5 3	38.0−	5.2	38.0+
	16	4 6	37.5+	5.0	38.5−
	32	3.6	39.0−	4.7	39.0

APPENDIX TABLE II

FLAVOR PANEL SCORES AND OFFICIAL GRADERS' SCORES OF BUTTER FROM PLANT B

Storage temperature, °F.	Storage time, days	Canned		Print	
		Panel	Graders	Panel	Graders
30	Initial	8.9	39.0—	8.7	39.0
	16	7.4	39.5+	6.9	39.0—
	32	7.2	39.5	6.9	39.0+
	64	7.5	39.0	6.4	36.0
	128	7.6	39.0—	4.2	38.0
	192	7.4	37.0	2.6	—
	256	6.3	37.0	3.8	—·—
	321	6.9	39.0—	3.4	36.0
	385	4.8	—	0.0	—
50	Initial	8.9	39.0—	8.7	39.0
	4	6.9	39.0	7.2	39.0
	8	7.1	39.0	7.4	38.5
	16	7.4	39.5—	5.9	39.0+
	32	5.1	38.0	4.5	37.0
	64	6.6	39 0	2.3	36.5
	96	4.6	38.0	1.5	36.0
	128	6.3	39.0	1.5	36.0
	192	6.5	38.0	1.2	—
	256	5.5	37.0	0.0	—
70	Initial	8.9	39.0—	8.7	39.0
	0.5	7.8	38.5	7.8	39.0
	1	7.7	39.5	7.7	39.0
	2	7.1	39.5	7.7	39.0
	3	7.5	39.5—	6.4	39.0
	4	6.9	39.0	6.9	39.0—
	8	6.1	37.5	7.2	37.5
	16	6.7	39.0	5.1	38.0—
	32	4.4	35.0	2.8	36.0
	64	2.5	35.5	1.0	36.0
	128	3.3	—	2.6	—
90	Initial	8.9	39.0—	8.7	39.0
	0.25	7.3	38 5	7.2	39.0—
	0.5	7.0	38.0	7.2	38.0
	1	6.5	38 5	6.9	38.0
	2	6.5	38.0	6.1	38.0
	3	6.0	38.0+	4.8	38.0—
	4	6.0	39.0—	5.2	38.0
	8	6.5	37.5	4.1	37.0
	16	5.3	38.5	3.8	37.0—
	32	5.2	39.5+	4.2	39.5—

EFFECT OF PROCESSING AND STORAGE ON THE QUALITY OF GELOSE FROM IRISH MOSS *(CHONDRUS CRISPUS)*

By D. MacDougall

EFFECT OF PROCESSING AND STORAGE ON THE QUALITY OF GELOSE FROM IRISH MOSS *(CHONDRUS CRISPUS)*[1]

By D. MacDougall[2]

Abstract

Irish moss (*Chondrus crispus*) from Canada's east coast was used to study possible improvements in the production and storage of gelose. The optimum pH for the extraction of gelose from Irish moss was between 6 and 7. Fine leaf particles, which prevent rapid filtration, were readily removed by supercentrifuging. Comparison of drum drying and drying from the frozen state showed that the former caused a marked lowering of viscosity but had little effect on suspending power or jelly strength. The logarithm of the viscosity determined by means of a MacMichael viscosimeter was related directly to the concentration; therefore, viscosity measurements can be corrected for the moisture content of the samples. The effect of storage temperature on deterioration was greater than the effect of relative humidity. Samples stored at 0° and 40° F for 12 weeks showed no significant changes in viscosity or suspending power, whereas those stored at 80° and 120° F. deteriorated very rapidly. Material was vacuum ice-dried to 2% moisture without damage but the quality decreased when the extracts were stored at high temperatures

Introduction

The principal use of the water soluble carbohydrates of Irish moss (*Chondrus crispus*) is in the dairy industry where it serves as a suspending agent in the manufacture of chocolate milk   It also finds considerable use in the pharmaceutical industry as a stabilizer for emulsions and suspensions. In addition to these it serves as a sizing agent in the textile industry, a clarifying agent in the manufacture of beer, a thickener for cold water paints, and a gelling agent in desserts.

Prior to 1939, the bulk of the Irish moss (*C. crispus*) used on this continent was imported from Europe. When this source of supply was cut off by the European war, agar-agar from Japan was substituted where possible   The entry of Japan into the war forced Canada to seek domestic sources of gelling material. Irish moss was available in quantity on Canada's east coast and the processing of this product was examined in these laboratories (2, 6). However, some commercial processors noted that stored dried extracts deteriorated in certain attributes. This paper describes an examination of some of the processing operations and storage conditions that might have affected storage stability.

Analytical Methods

Moisture was determined by drying in air for 16 hr. at 212° F. pH was determined with a Leeds and Northrup pH meter.

A number of methods were tried to establish possible criteria of quality. Reducing sugars (5), free sulphate (8, p. 613), and determination of the gelose content of extracts with benzidine (3) all showed little difference between fresh and stored extracts. Fluorescence values were also useless. However, the commonly used criteria, suspending power, viscosity, and jelly strength, were all found to be useful. The relation of these properties to the uses listed above is obvious.

Suspending Power

The minimum weight, in grams, of dried gelose extract required to give perfect suspension of cocoa in milk was used as an index of suspending power. In the procedure used, various amounts of the dried extracts were weighed into quart cans and 500 gm. milk (2% butterfat), 31 5 gm. sugar, and 5 8 gm. cocoa were added. The containers were closed with screw caps, blocked in a churn, and rotated end over end at about 20 r.p.m. for five minutes at room temperature to mix the ingredients. The churn was then filled with boiling water and rotated for 20 min. The hot water, now about 158° F., was removed, and the milk cooled by two five-minute churnings in water at about 41° F. The contents of the cans were then strained through 80 mesh screens (U.S. Bureau of Standards) into pint milk bottles which were held for 16 to 20 hr. at 40° F. and then for four hours at room temperature.

A suggested alternative method for determining suspending power, described by Rice (7), was examined and found unsatisfactory. Repeated attempts failed to give reproducible results. Rice states that the method is inaccurate in the presence of neutral salts and this may explain some of the difficulty, as these tests were made on commercial material whereas Rice used highly purified extracts. The method is even more cumbersome than the chocolate milk procedure because of the larger number of laboratory manipulations, and numerous attempted modifications to make it more easily applicable were unsuccessful.

Other tests of suspending power were tried but bore no relation to the chocolate milk test. These included: gold numbers (4, p. 58); stabilization of a standard ferric oxide solution against the precipitating action of sodium sulphate; and measurement of the precipitated cocoa in conical-bottomed centrifuge tubes.

Viscosity

The viscosity was determined in a MacMichael viscosimeter with the cup rotating at 20 r.p.m. A portion of dried moss extract (4 00 gm) was dusted into 200 ml. of distilled water and the mixture was heated in a boiling water bath for 10 min. with stirring. The sol was made up to its original weight with distilled water, and was cooled to 140° F. The viscosity was then determined. All viscosity values are expressed in MacMichael units

The moisture content of air-dried extract varied from 2 to 30% depending on the relative humidity at which the material was stored. It was, therefore, necessary to correct for the moisture content of each sample so that the values

for all determinations could be brought to a common basis. The concentration–viscosity relation was investigated on several different lots of material. Statistical analysis of the results indicated that a correction factor of 0.3940 units of log viscosity for each additional gram of dry matter was generally applicable. This formula was further tested on several lots of commercial material, with the results shown in Fig. 1.
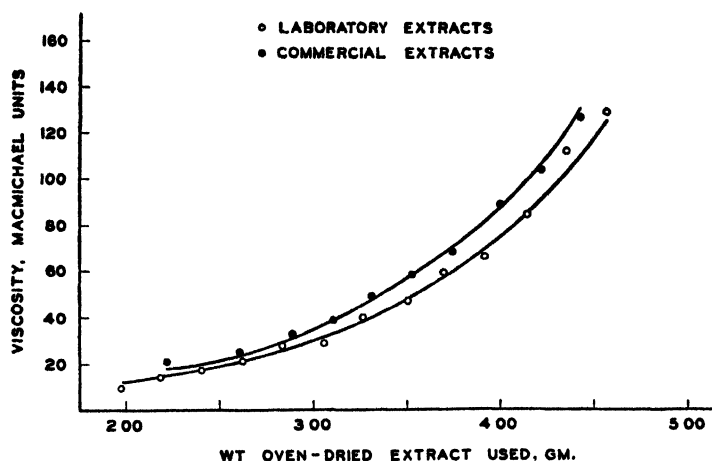

FIG. 1. *The relation between viscosity and concentration of oven-dried extracts of Irish moss.*

Jelly Strength

The method of preparation of the material was similar to that for the viscosity test except that 1.50 gm. of dried extract and 100 ml. of distilled water were used and the sample was cooled to 40° F. and held there for 16 hr. Jelly strengths were determined at this temperature with a Hall gelometer. All values are expressed in Hall units, which vary inversely with the jelly strength.

The Effect of Some Processing Procedures on Quality

pH of Extraction Medium

Buffer solutions (10 liters) 0.05 M with respect to phosphate and ranging in pH from 5 3 to 8.0, prepared as described by Clark (1, p. 81), were used to extract 300-gm. samples of fully bleached Irish moss. The extracts were dried from the frozen state (2), ground, and tested. Results of the experiment are contained in Table I. pH had little effect on the percentage of gelose in the extracts obtained, but because the more acid solutions gave less viscous sols, separation from the leaf residue was more complete and higher yields were obtained from these. There was little effect of pH on suspending power. Buffer solutions of pH 5 3 and 5 6 resulted in a material with poor viscosity when made into sols, and solutions of pH 7.4, 7.8, and 8.0 gave materials that formed gels too weak to be measured. Therefore, the pH for optimum viscosity lies between 6 and 7.

TABLE I

pH of extraction solution	Yield, %	Solids in extract, %	Minimum conc. giving complete suspension, gm.	Viscosity of product, MacMichael units	Jelly strength, Hall units	pH of jelly
5.3	43	2.2	0.28	3	2.2	6.0
5 6	51	2.4	0.27	4	2.1	6.1
6.0	38	2.1	0.28	8	1.9	6 2
6.3	28	1.8	0.26	12	1.7	6.3
6.5	28	1.8	0.28	9	2 0	6.5
6.7	29	1 8	0.30	14	2.2	6.7
6.8	26	1.8	0.30	17	1.8	6.8
7.0	32	1.9	0 30	16	2 4	7.0
7.2	26	1.8	0 35	17	2.1	7.1
7.4	25	1 8	0.30	17	—	7.4
7.8	27	2.0	0 34	24	—	7.6
8.0	28	1.9	0.30	11	—	7.6

Separation of Leaf Fragments

Fulton and Metcalfe (2) suggested the use of a countercurrent extractor to increase the solid content of the gelose solution. However, they were obliged to extract twice to obtain a satisfactory yield of gelose, because, if the leaves were filtered under pressure, many fine leaf fragments remained in the extract and these prevented subsequent filtration. Basket centrifuging did not remove them.

However, the Sharples supercentrifuge was capable of removing a sufficient number of the tiny leaf fragments to render filtration easier. The present procedure consisted of two steps: drainage through a 40 mesh screen (U.S. Bureau of Standards), followed by air pressure filtration (10 lb. per sq. in.). As much of the clear liquid as would readily drain from the leaves was removed first and set aside. Then the portion obtained by pressure filtration was heated to about 194° F. and blown through the supercentrifuge at a rate of 0.3 liter per min. The centrifuged liquid was then combined with the drained liquid before treatment with charcoal and filter-aid (Johns-Manville No. 545). The resulting solutions were clear, odorless, and tasteless, and the yield was as high as that with the countercurrent extraction method (2).

Drying

Since commercially available moss extracts are usually dried on steam heated drums, a comparison was made between material prepared by the freeze drying method developed in these laboratories (2) and material prepared on a laboratory double-drum drier (steam pressure in the drums, 50 lb. per sq. in., gauge). For both tests the starting material consisted of a sol of Irish moss gelose containing potassium chloride and with a solids concentration

of 4%, obtained by adding previously dried extract. At this concentration, the material dried from the frozen state had fewer ice crystals embedded in the partially dehydrated sheet and it was unnecessary to place crushed ice on the jelly to prevent supercooling.

The results, given in Table II, show that material dried from the frozen state is very little better than drum dried material as a suspending or jelling agent, but that the former produces sols with much greater viscosity. This

TABLE II

QUALITY TESTS ON DRUM- AND FREEZE-DRIED EXTRACTS OF IRISH MOSS GELOSE, STORED AT 40° F.

Drying method	Storage time, days	Moisture, %	Jelly strength, Hall units	Suspending power, gm.	Viscosity, MacMichael units
Drum	0	12.0	1.20	0.22	15.5
	15	11.5	1.25	0.20	16
Freeze-dried	0	16 0	1.25	0.17	59
	15	15.0	1 30	0.19	44

finding, together with the results given under "pH of Extraction Medium", indicates that suspending power is a relatively stable property of Irish moss gelose, and that viscosity values are not related to indices of suspending power.

The Effect of Storage on Quality

In this experiment both dried extracts prepared in this laboratory and samples of commercial material were studied. Samples were stored at 0°, 40°, 80°, and 120° F. in relative humidities of 0%, 30 to 40%, and 70 to 75%. Each batch was sampled after 1, 2, 4, 8, and 12 weeks of storage.

In view of the variability of the data on viscosity and suspending power, they were transformed to logarithms, plotted against logarithms of time in weeks, and the slopes b of the best fitting straight lines were determined. The regression coefficients and their necessary differences are shown in Table III. There was no significant decrease in the viscosity of the samples stored at 0° and 40° F. However, there was a significant decrease in the viscosity of all samples stored at 80° and 120° F. In the samples stored at 120° F. the relative humidity had significant effects on the rate of viscosity loss. The suspending power did not change during storage at the lower temperatures, the only significant difference being at 120° F. and high relative humidity.

After 12 weeks' storage the reducing sugar (5), free sulphate (8, p. 613), and final moisture contents of each of the storage batches were determined. The pH of 2% solutions of material stored at 0° and 120° F. was also determined. No reducing sugar was found in any of the samples. The results of the remaining measurements, given in Table III, show that free sulphate was

TABLE III

THE QUALITY OF SAMPLES OF IRISH MOSS GELOSE STORED FOR 12 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Criteria	Storage Temperature, °F.	0% R.H.*	30–40% R.H.*	70–75% R.H *	Necessary difference, 5% level
Average decrease of log viscosity per log week, b	0	0 08	0 00	0 04	
	40	0 02	0 01	0 10	0.11
	80	0 19	0 19	0 26	
	120	0 47	0 62	0 76	
Average increase of log suspending power index per log week, b	0	0 05	0 04	0 03	
	40	0 04	0 01	0 01	0.16
	80	0 06	0 10	0 11	
	120	0 12	0 12	0 17	
Final pH, done on 2% solutions	0	5 4	5 5	6 1	
	40	—	—	—	
	80	—	—	—	—
	120	4 9	5 2	5 0	
Final free sulphate content, p.p.m.	0	Trace	Trace	0	
	40	Trace	0	Trace	
	80	Trace	0	0	—
	120	2,690	3,070	4,560	
Final moisture content, %	0	8 5	13 8	23 9	
	40	3 7	12 0	22 2	
	80	1 1	13 9	21 3	
	120	1 0	13 2	21 9	

* *Relative humidity.*

found only in material stored at 120° F., and that solutions of material stored at 120° F. were more acid than solutions of material stored at 0° F. With the exception of samples stored at 0% relative humidity and at the lower temperatures, moisture content had apparently reached equilibrium during the 12 week storage period. Relative humidity had important effects on moisture content and should preferably be kept below 50% relative humidity. In general, storage temperatures of 80° F. or higher caused some deterioration while temperatures of 40° F. or lower were satisfactory.

Samples of commercial material were stored under atmospheres of air, oxygen, and nitrogen at 140° F. and at relative humidities of 0% and 70 to 75%. These were tested at various intervals and the results, given in Table IV, show that there was little difference in the rates at which the samples deteriorated.

TABLE IV

EFFECTS OF STORAGE UNDER DIFFERENT ATMOSPHERES AT 140° F. ON IRISH MOSS GELOSE

Atmosphere	0% R.H.			70 to 75% R.H.		
	Storage time, days	Viscosity, MacMichael units	Suspending power, gm.	Storage time, days	Viscosity, MacMichael units	Suspending power, gm.
Air	0	22.5	0.25	0	22.5	0.25
	3	13.5	—	6	4.5	—
	10	6.0	0.40	12	3.5	—
	20	6.0	—	17	2.5	—
	34	4.0	0.60	20	2.5	0.65
Oxygen	0	22.5	0.25	0	22.5	0.25
	3	12.5	—	6	4.5	—
	10	8 0	—	12	2 5	—
	14	6.5	—	17	2.5	—
	20	6.5	0.30	20	1.0	No suspension
	34	4.0	—			
Nitrogen	0	22.5	0 25	0	22.5	0 25
	3	17.0	—	3	11.5	—
	10	10 0	—	10	4 0	—
	14	8.5	—	14	2 5	—
	20	8.5	0.40	20	2.0	No suspension
	34	2.5	—	34	0	—

It was thought that loss of viscosity at high temperatures might be due to the initial moisture contents of the samples (about 10%). This was not evident in the present work as it required approximately two weeks to lower the moisture content of samples stored at 120° F. and 0% relative humidity to 2%. By that time a considerable amount of deterioration had already taken place. Accordingly, samples of commercial material were dried at 104°, 122°, and 140° F. under partial vacuum (25 in.). The materials were tested after 1, 2, 4, 6, 24, and 48 hr. of drying. The results in Table V show a marked decrease in viscosity as moisture was removed from the sample.

TABLE V

EFFECT OF DRYING TEMPERATURE ON VISCOSITY OF EXTRACTED GELOSE

Drying time, hr.	104° F.		122° F.		140° F.	
	Viscosity, MacMichael units	Moisture, %	Viscosity, MacMichael units	Moisture, %	Viscosity, MacMichael units	Moisture, %
Original material	80*	10.0	80	10.0	80	10.0
1	78	9 5	81	7.9	81	8.0
2	78	8.7	80	6.0	72	7.0
4	74	7.1	65	4.3	69	5.3
6	—	—	67	4.3	63	4.1
24	74	4.0	67	3.8	47	2.8
48	67	4.5	—	—	—	—

' *All viscosity values were corrected for moisture content of sample.*

When high temperature drying was found to be undesirable, a sample of dried extract was held for two weeks at 0° F. under partial vacuum (25 in.). The product then had 2% moisture and a viscosity equal to that of the original material. Portions of the original material and this dried product were then stored over phosphorus pentoxide at 120° F. The moisture content of both materials gradually decreased to zero and both decreased in viscosity. It appears, therefore, that deterioration at high temperatures is not due to the initial moisture content of the material.

Discussion

The present work shows that the suspending power and the viscosity of sols from Irish moss gelose prepared by different procedures are not related. Gelose finds its main use as a suspending agent in chocolate milk, and for this purpose the method of preparation does not appear to be important, since suspending power is a relatively stable property. For other uses (e.g., thickening agent in pharmaceuticals, cold water paints, etc.) where high viscosity is desired, the method of preparation may be extremely important, since viscosity is substantially affected by the method of preparation. High temperature drying appears to be particularly detrimental. The results indicate that products with high viscosity can be obtained by drying and concentrating by a freezing process, and that these extracts should preferably be stored at temperatures below 40° F.

While these experiments were not designed to determine the fundamental nature of the deterioration, it might be observed that, since the deterioration was more pronounced in samples of high moisture content and was associated with an increase in free sulphate, the changes resulting in a reduction of viscosity may be of a hydrolytic character.

Acknowledgments

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LIQUID AND FROZEN EGG

IV. REPRODUCIBILITY OF MEASUREMENTS OF REDUCING SUGAR IN FROZEN EGG[1]

By J. W. Hopkins[2] and Ruth M. Trevoy[2]

Abstract

Analysis of 16 test samples in three collaborating laboratories indicated the desirability of initial standardization and periodic checking of reagents and of both sampling and analytical technique to ensure consistency of routine results. Average glucose content of a carlot of 1250 38-lb. containers might be determined with a standard error of the order of ± 10 mgm. per 100 gm. egg by a single analysis of each of two independent composite samples each obtained by combining and thoroughly mixing single 'cores' taken from 15 randomly chosen containers. This would also enable a running check to be kept on both the average and variance of the reported glucose contents by, e.g., 'control chart' methods. Precision of test results would be improved most effectively by increasing the number of individual containers sampled rather than the number of chemical analyses.

Object and Methods

Considerable quantities (amounting in 1947 to some 20,000,000 lb.) of eggs accumulated during the season of peak Canadian production are commercially preserved for use in the bakery and other trades by removing them from the shell, mixing yolks and whites, and freezing the resultant mixture in bulk containers holding 36 to 40 lb. Objective criteria for assessment of the quality of this frozen product are desirable, and it has been suggested (6) that reducing sugar content might be one such criterion, measurable in either plant or local consulting laboratories. Reproducibility within and between laboratories of this measurement has accordingly been studied with the results now described. Three Ottawa laboratories collaborated in the investigation. These were located in the Division of Chemistry, Science Service, Department of Agriculture (J. T. Janson); Food and Drug Division, Department of National Health and Welfare (L. I. Pugsley); and Division of Applied Biology, National Research Laboratories (J. A. Pearce). Test material consisted of 16 38-lb. pails of commercial frozen egg drawn from current production of plants in Montreal, Toronto, Ottawa, and Winnipeg. These will hereafter be designated by the letters A to P, while the collaborating laboratories will be denoted by the numbers 1, 2, and 3, which were allotted to them at random.

Fig. 1 illustrates schematically the procedure followed in apportioning and subsampling the test material. Apportioning was done in the National Research Laboratories. The contents of each pail was first divided into six 6⅓ lb. portions by vertical slicing along equidistant radii of the top surface.

The resulting portions were then kept in −40° F. storage pending distribution to the collaborators as scheduled in Table I. It may be observed from this schedule that the test extended over 16 working days, each laboratory receiving two of the above-mentioned 6⅓ lb. portions, identified only by code number, for subsampling and analysis on each day. The schedule for each laboratory

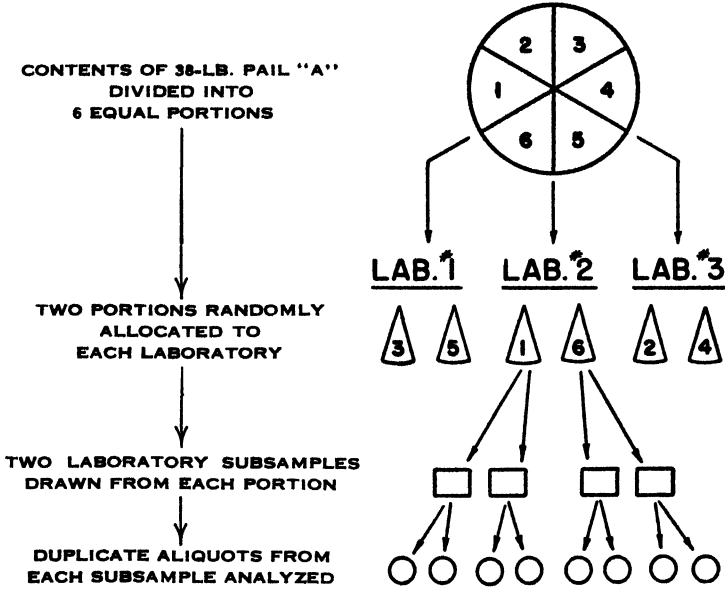FIG. 1. *Schematic representation of apportionment and subsampling of test material.*

TABLE I

SCHEDULE OF DISTRIBUTION OF TEST PORTIONS

Day of test	Test material (designation A–P)					
	Laboratory 1		Laboratory 2		Laboratory 3	
	First	Second	First	Second	First	Second
1	B	P	E	H	M	F
2	N	K	G	M	C	A
3	F	O	N	B	P	O
4	J	G	P	A	E	B
5	A	M	F	C	L	J
6	P	B	H	E	F	M
7	C	E	O	K	I	D
8	I	H	L	I	H	K
9	G	J	A	P	B	E
10	M	A	C	F	J	L
11	L	D	D	J	G	N
12	D	L	J	D	N	G
13	E	C	K	O	D	I
14	K	N	M	G	A	C
15	O	F	B	N	O	P
16	H	I	I	L	K	H

was balanced in two respects. Firstly, over the 16-day period as a whole, one portion from every pail was analyzed as a 'first' and one as a 'second' daily sample, thus facilitating the detection of any consistent diurnal trend in results. Secondly, portions from eight pairs of pails were analyzed on two different days in each laboratory (e.g., from N and K on the second day and from K and N on the fourteenth day in Laboratory 1), so that marked differences between days affecting any or all laboratories might likewise be distinguishable from the inter-portion sampling variance. Apart from these limitations however, the order of distribution of the various portions was determined entirely from tables of random numbers (4).

From each submitted portion, the laboratories withdrew two independent small subsamples, which were carried through all further stages separately but concurrently. After the removal of protein in the manner specified by Folin and Wu (5, p. 416), the reducing sugar in 2 gm. of defrosted material was measured by the Shaffer–Hartmann titration procedure (5, pp. 437–438) and expressed as mgm. glucose per 100 gm. egg. Duplicate aliquots were titrated. All determinations in any one laboratory were made by a single technician in accordance with standardized instructions covering 21 specific items of procedure.

Results

Intra-laboratory Variance

The test provided information respecting the variability within each laboratory of aliquots from the same subsample, of subsamples from the same portion, and of portions from the same pail. This was examined statistically by Fisher's analysis of variance procedure (3).

Variance between duplicate aliquots from the same subsample, estimated from 64 comparison-pairs in each laboratory, differed as shown in Table II.

TABLE II

ESTIMATED INTRA-LABORATORY STANDARD DEVIATIONS

(Mgm. glucose per 100 gm. egg)

Source of variance	Standard deviation, mgm.		
	Lab. 1	Lab. 2	Lab. 3
Aliquots	5	2	11
Subsamples	19	9	9
Test portions	16	16	16

In Laboratory 1 it gave rise to a standard deviation of ± 5 mgm., this being ±1.4% of the average of 356 mgm. glucose per 100 gm. egg reported there for the test as a whole. In Laboratory 2 the aliquot standard deviation was ± 2 mgm., which was ± 0.6% of this laboratory's average of 354 mgm.,

while in Laboratory 3 it was ± 11, amounting to ± 3.5% of the average of 319 mgm. The recorded aliquot standard deviation of Laboratory 2 was thus less than half, while that of Laboratory 3 was more than double, that of Laboratory 1.

Subsamples drawn from the same portion, on the other hand, were substantially less variable in both Laboratories 2 and 3 than in Laboratory 1. After allowing for the variance in aliquots described above, subsampling was estimated (1, 2), from 32 comparison-pairs in each instance, to have given rise to a standard deviation of ±19 in Laboratory 1, but of only ±9 mgm. in Laboratories 2 and 3.

As the test portions resulting from the initial division of the contents of each pail were allocated in an objectively random manner, variability in the results of all three collaborators due to any heterogeneity of such portions should have been of the same order. This was in fact the case. The net variance between portions after allowing for that in aliquots and in subsamples deduced above, estimated from 16 comparison-pairs in each laboratory, corresponded to a standard deviation of ± 16 mgm. glucose per 100 gm. egg. These differences in the results for portions from the same pail were apparently entirely random in character, no consistent discrepancy in average or variance between days, or between first and second sets of determinations made on the same day being detectable when the laboratories were considered either individually or collectively.

Inter-laboratory Variance

Table III and Fig. 2 provide a comparison of the results reported by the three collaborators. Each value listed is an average of two aliquots from two subsamples from two portions from each test pail, viz., of eight titrations in all. The concentration of experimental material in the upper part of the range of variation was presumably representative of current production but was not ideal for correlation studies. Agreement between Laboratories 1 and 2 appeared to be basically good, the individual and collective departures from equality, represented graphically by the distance of the hollow circles from the broken diagonal line in Fig. 2, being no more than the expectation computed from the previously estimated variance of test portions, subsamples, and aliquots. Laboratory 3's results on the other hand were in the aggregate significantly lower, and there was some suggestion that this discrepancy widened as the glucose content of the test material increased, corresponding pail averages for Laboratories 1 (x_1) and 3 (x_3) being related by the conversion equation (7)

$$x_3 = 100.1 + 0.616x_1$$

illustrated by the continuous line in Fig. 2. In the absence of additional results for the range 200 to 300 mgm., however, this may be subject to considerable revision, the standard error of the factor 0.616 being of the order of ±0.15.

TABLE III

TEST RESULTS REPORTED BY COLLABORATORS

Test material	Average reducing sugar reported (as mgm. glucose per 100 gm. egg)		
	Laboratory 1	Laboratory 2	Laboratory 3
A	381	381	318
B	336	373	333
C	376	372	297
D	197	212	216
E	349	306	296
F	368	343	357
G	363	359	333
H	383	341	300
I	382	356	319
J	367	378	329
K	351	345	323
L	337	358	357
M	354	357	339
N	377	387	330
O	411	396	331
P	358	404	329
Average	356	354	319
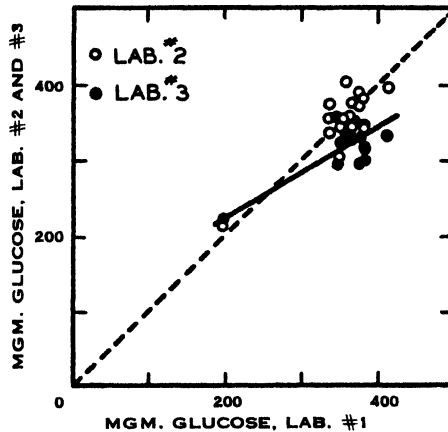
FIG. 2. *Graphical comparison of glucose contents of 16 test materials reported by three collaborating laboratories.*

Conclusions

If current stocks were to be tested, practical considerations would require estimation of the average reducing sugar content of carload shipments of 1250 or more pails from single core samples taken from about 2% and amalgamated to form composites for analysis in local consulting laboratories. Subsequent comments relate to these imposed conditions.

The foregoing experiment of course strongly suggests that initial standardization, and periodic checking, of reagents and of both sampling and analytical technique would be desirable to ensure consistency of grading in all localities.

The over-all standard deviation of results for carlots obtained as outlined would reflect inter-pail as well as intra-pail variability. The 16 pails A–P considered above were characterized by a net variance (after allowing for sampling, subsampling and aliquot fluctuations) of 1882, corresponding to a standard deviation of ± 43 mgm. glucose per 100 gm. egg. This figure may exceed the actual variability within carlots from a single plant, but makes possible an approximate estimation of the level of precision likely to be attained in practice. If the variance of inter- and intra-pail sampling, of laboratory subsampling, and of individual aliquots be denoted by v_1, v_2, v_3, and v_4, and if it be supposed (a) that a carlot comprises 1250 pails of which a random 2.4% are subjected to single-core sampling; (b) that the resulting 30 cores are combined to form two thoroughly mixed 15-core composites for independent analysis; (c) that from each of these composites a laboratory subcomposite is formed by withdrawing and again thoroughly mixing three independent laboratory subsamples; and (d) that one chemical determination is made on a 2 gm. portion of each such subcomposite: then the standard error to be expected in the average of the two determinations thus made on material from each carlot would be

$$\pm [(v_1 + v_2)/30 + v_3/6 + v_4/2]^{\frac{1}{2}}.$$

With $v_1 = 1882$ as above, $v_2 = 249$ as in the average for Laboratories 1, 2, and 3, $v_3 = 77$ as in Laboratories 1 and 2 and $v_4 = 29$ as in Laboratory 1, this would amount to roughly

$$\pm (63 + 8 + 13 + 15)^{\frac{1}{2}},$$

i.e., of the order of ± 10 mgm. per 100 gm. egg. Hence, if for example a grade specification of not less than 300 mgm. per 100 gm. were established and shipments were inspected in the above way, a carlot for which the average was actually 275 mgm. or lower should have less than 1 chance in 100 of being accepted, while one for which the true average was 325 mgm. or higher should stand less than 1 chance in 100 of being degraded. The indications are that this margin of buyer's and seller's risk would be most effectively reduced by increasing the proportion of pails sampled rather than the number of laboratory analyses.

Of course, this estimate of likely precision must be regarded as tentative, as its major component, inter-pail variance, is only approximately known. However, in the event of the procedure being adopted for routine grading, analysis of two independent core-composites from each carlot would soon provide more definite information on this point, as well as enabling a running check to be kept on both the average and variance of the reported glucose contents by 'control chart' or other methods (8). Duplicate analyses would permit a similar running check to be kept on laboratory technique, but would add to laboratory costs without effecting a corresponding increase in the over-all precision of the results.

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Action of Sulpha Compounds, Antibiotics and Nitrite on Growth of Bacteria in Fish Flesh

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ABSTRACT

Sodium nitrite (0.02%) inhibited growth more than sulphathiazole (0.001-0.002%) or sulphanilamide (0.002%), but penicillin G (5-75 I.U. per 100 g.) and streptomycin (100-10,000 "S" units per 100 g.) had no important effect.

These experiments were undertaken in order to establish the relative effectiveness of incorporated sulphanilamide, sulphathiazole, penicillin, streptomycin and sodium nitrite in retarding development of the mixed natural bacterial flora of fish flesh. It was previously found that, under certain conditions, nitrites exerted marked bacteriostatic action both in fish flesh (Tarr and Sunderland 1939a; Tarr 1940) and in culture media (Tarr 1940, 1942). Recent experiments have shown that ices containing quite small amounts of sulphathiazole or sulphanilamide (0.01 to 0.02%) delay bacterial development in flesh of whole iced fish (Tarr 1946, 1948) and have also verified previous findings (Tarr and Sunderland 1939b, 1940) that ice containing 0.1% of incorporated sodium nitrite is normally quite effective in this respect.

EXPERIMENTAL

The fresh fish employed were thoroughly scrubbed in running tap water in order to avoid possible heavy bacterial contamination of the flesh during subsequent filleting. The skinned fillets were passed through a Universal food chopper, the pH of a representative portion of the comminuted material being determined by means of a Beckman meter. A direct bacterial count (Tarr 1943) made of the minced flesh showed that initially it contained less than 100,000 bacteria per gram in all instances. North's stain (North 1945) was used instead of the usual Loeffler's stain in making the direct counts. Tests with both fish and meat have shown that somewhat higher counts are normally obtained with the North stain, and that the background of flesh material is less heavily stained than with Loeffler's stain. The North stain appears to deteriorate rather quickly.

Sulphathiazole (Merck), Penicillin G (Winthrop), Sulphanilamide (Eastman Kodak), Streptomycin hydrochloride (Merck) and sodium nitrite (C.P.) were employed. With the exception of sulphathiazole, which was dissolved in warm

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water, the compounds were dissolved in water at room temperature immediately prior to use. These bacteriostats were tested as follows.

Comminuted flesh (95 g.) was transferred to each of several 250-ml. sterile, covered glass beakers, and 5 ml. of water (in controls) or of aqueous solutions of

TABLE I. Effect of sodium nitrite, sulphathiazole, sulphanilamide and penicillin, on growth of bacteria in red spring salmon and lingcod flesh.

Treatment	Bacteria (millions per g.) by direct count				
	Red spring salmon (initial flesh pH, 6.2) after			Lingcod (initial flesh pH, 6.5) after	
	4 days	7 days	10 days	4 days	7 days
Controls	2	400	3700	6.2	950
0.02% sodium nitrite	0.13	4.6	99	1.2	68
0.001% sulphathiazole	0.80	20	710	3.0	40
0.002% sulphathiazole	0.90	21	870	3.5	49
0.002% sulphanilamide	0.64	40	1200	4.0	84
5 I.U. of penicillin G*	1.7	310	2000	3.1	610
20 I.U. of penicillin G*	2.2	390	2900	4.1	780
75 I.U. of penicillin G*	1.4	230	3800	5.4	870

*International units per 100 g. of fish flesh.

the bacteriostats in concentrations sufficient to cause the minced flesh to contain the quantities of these as recorded in tables I and II was uniformly incorporated by means of a heavy glass rod. The samples were stored at 0°C. At intervals the flesh was mixed thoroughly and 7.5-g. samples were removed and direct bacterial counts made.

TABLE II. Effect of sodium nitrite, sulphathiazole and streptomycin on growth of bacteria in red spring salmon and halibut flesh.

Treatment	Bacteria (millions per g.) by direct count					
	Red spring salmon (initial flesh pH 6.0) after			Halibut (initial flesh pH 6.15) after		
	7 days	11 days	16 days	7 days	11 days	16 days
Controls	0.27	260	4500	0.69	69	2700
0.02% sodium nitrite	<0.1	0.29	32	<0.1	0.95	12
0.001% sulphathiazole	<0.1	13	1600	<0.1	4.1	1200
100 "S" units of streptomycin*	0.27	350	3200	0.75	360	1900
1000 "S" units of streptomycin*	0.40	143	5100	0.45	220	4000
10,000 "S" units of streptomycin*	0.13	53	4000	<0.1	66	1500

*Streptomycin hydrochloride units per 100 g. of fish flesh.

The results of typical experiments with red spring salmon (*Oncorhynchus tshawytscha*), halibut (*Hippoglossus stenolepis*) and lingcod (*Ophiodon elongatus*) are recorded in tables I and II. In the lingcod samples, bacterial counts were not made after 10 days' storage because all the samples were obviously very stale. It will be seen that with this fish, bacteriostats which were effective with salmon and halibut afforded only a slight protection against bacterial spoilage. As suggested in previous publications (Tarr 1940, 1948), this may be due to the higher pH of lingcod flesh. Sodium nitrite was the most effective of the bacteriostatic substances investigated for salmon and halibut and also quite effective for lingcod. Sulphathiazole afforded a considerable degree of protection of these fish against bacterial spoilage, and appeared to be somewhat more active than sulphanilamide. No important decrease in the rate of bacterial increase in fish flesh was observed following incorporation of penicillin or streptomycin.

Organoleptic examination of certain of the samples showed that the nitrite-treated flesh invariably had a better colour and odour than the untreated flesh, and that this bacteriostat delayed the fading of the red astacin pigments of salmon. Sulphathiazole and sulphanilamide did not appear to prevent this fading but considerably delayed the development of unpleasant odours in the stored flesh, even with lingcod. Neither penicillin nor streptomycin was effective in delaying organoleptic spoilage.

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Control of Rancidity in Fish Flesh
II. Physical and Chemical Methods

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ABSTRACT

Storage of frozen fish in high partial pressures of carbon dioxide or nitrogen effectively pre-vented fat oxidation and organoleptically detectable rancidity. Carbon dioxide stored fish de-veloped an undesirable flavour not observed in that stored in nitrogen. Ice glazes formed from water or ethyl gallate solutions were about equally effective in delaying fat oxidation in frozen salmon steaks or fillets, but considerably less effective than 1-ascorbic acid or sodium 1-ascorbate glazes. Immersing salmon steaks or fillets in 1-ascorbic acid or sodium 1-ascorbate solutions strongly inhibited fat oxidation. 1-Ascorbic acid and closely related compounds were as effective, or more effective, than ethyl gallate or several other gallic acid derivatives for frozen fish. When 1-ascorbic acid was added to fish flesh its concentration fell rapidly at first, and then very slowly in the frozen fish.

The comparative value of a number of incorporated chemical antioxidants in delaying the onset of rancidity in indigenous fats of fish flesh was previously determined (Tarr 1945, 1947). The present paper describes experiments in which physical and chemical methods, alone or combined, have been investigated in order to assess their ability to retard fat oxidation in fish flesh.

Physical methods of retarding rancidity development in cold stored fish which are in use commercially include such common practices as the ice glazing of frozen whole single fish, or blocks of small fish, and the wrapping of fillets or steaks in certain moisture-proof papers. These methods are probably employed more with the object of preventing desiccation than of delaying fat oxidation, but there is little doubt that they do assist in retarding the development of rancidity.

EXPERIMENTAL

The methods of incorporating antioxidants into minced fish flesh, of freezing and storing samples, and of performing peroxide value determinations after chloroform extraction of the fat, were identical with those previously described (Tarr 1947). The 1-ascorbic acid content of fish flesh was determined as follows: A known weight (usually 100 to 200 g.) was blended for not more than 30 seconds with three times its weight of water, using a Waring Blendor. Fifty ml. of the resulting suspension was promptly mixed with 50 ml. of acetic-metaphosphoric acid solution, filtered through coarse paper, and 50 ml. of the clear filtrate then

titrated with sodium dichlorbenzenoneindophenol indicator. The solutions employed were prepared and standardized according to the official method of the Association of Official Agricultural Chemists (1945). It was found that, providing all procedures were carried out very rapidly in order to avoid oxidation of ascorbic acid, recoveries of from 99 to 100% of the theoretical amount could be realized when from 0.025 to 0.050% of this antioxidant was added to fish flesh.

STORAGE IN NITROGEN OR CARBON DIOXIDE

Preliminary tests with small samples of minced flesh from red spring salmon (*Oncorhynchus tschawytscha*), coho salmon (*O. kisutch*), pink salmon (*O. gorbuscha*), chum salmon (*O. keta*), black cod (*Anoplopoma fimbria*) and herring (*Clupea pallasii*), with and without 1% of incorporated sodium chloride, showed that storage in very high partial pressures of nitrogen or carbon dioxide gases entirely prevented fat peroxidation. Moreover, these trials showed that very great care had to be taken to prevent access of air to the containers, for a minute leak was sufficient to cause marked fat oxidation during long storage periods. In the following experiment, the results of which have been briefly described (Tarr 1946a), great care was taken to prevent diffusion of gas from, or into the containers used.

Three cast aluminum standard domestic pressure cookers were used for the gas storage tests, and since the cast aluminum was found to be quite permeable to gases such as carbon dioxide, the cookers and their lids were thoroughly impregnated with hot paraffin wax under pressure. Each lid was equipped with two brass gas cocks, and both these and the lid flanges were lubricated with a heavy laboratory-prepared rubber grease. The following fish were used:

Fresh herring. These were scaled, gibbed, washed and split.

Red spring salmon. An 8-lb. (3.6-kg.) fish which had been dressed, frozen, glazed and stored for about 4 months at approximately −28°C. was defrosted in cold running water and filleted. The fillets were skinned, and each was cut into eight pieces of roughly the same weight.

Coho salmon. Two 5-lb. (2.3-kg.) frozen fish having approximately the same storage history as the red spring salmon were cut up so that each fish yielded eight small skinned fillets.

Pink salmon. One 5-lb. (2.3-kg.) frozen fish having approximately the same storage history as the red spring salmon was cut up so that eight small skinned fillets were obtained.

Lemon sole (Parophrys vetulus). Eight medium-size fresh fish weighing about 1 lb. (0.5 kg.) each were filleted and the sixteen resulting fillets were skinned.

Small portions of each of several of the above types of fillets (brined and unbrined) were taken at this stage and wrapped and frozen as indicated below. These were defrosted immediately and were used to obtain the initial peroxide values (table 1). Half of the fillets from each fish were wrapped individually in "M.S.T." (moisture-proof, heat-sealing, transparent) cellophane. The remainder were lightly brined by immersing them for one minute in 10% sodium chloride solution at between 2 and 5°C. and then wrapped after draining on a wire screen for about 5 minutes at room temperature. The wrapped fillets and split herring

were packed into eight standard 5-lb. waxed fillet cartons so that two cartons containing the same selection of fish, brined and unbrined, could be employed for each of four types of storage test. The cartons were placed in an experimental "plate" freezer refrigerated with calcium chloride brine, and were frozen for 3 hours. They were stored overnight at about −28°C. and subjected to the following treatments carried out at −10°C.

(1) Two cartons, one each of brined and unbrined samples, were stored in air as controls.

(2) Two cartons were placed in an aluminum container connected to a high-vacuum pump and evacuated for 45 minutes to a pressure of about 0.1 mm. of mercury. Commercial carbon dioxide gas was then run into the container until a positive pressure equivalent to approximately 10 cm. of mercury resulted.

TABLE I. Effect of storage in nitrogen or carbon dioxide gases on development of rancidity in fish samples stored at −10°C., as shown by peroxide value.

Treatment		Herring		Red spring salmon		Coho salmon		Pink salmon	Sole	
After days (no.)		40	98	40	98	40	98	40	40	98
Stored in air	Unbrined	7.5	18 4	4 7	10.0	14 0	46.9	43 0	---	45 0
	Brined	9 8	76 3	10 1	30.2	5 6	7.7	16 0	5.8	11.4
Vacuum then CO_2 added	Unbrined	0 1	0 8	0 0	0 4	0 1	0 2	0 0	0.0	1 8
	Brined	1 2	1 5	0.4	0 6	0 2	0 2	0 9	0 0	2 0
Vacuum, then nitrogen added	Unbrined	0.3	0 8	0.1	0.5	0 1	0 7	0 0	0 0	2.8
	Brined	1 1	1 5	0 4	1.4	0 1	0 4	0 6	0 0	3.1
Flushed with CO_2	Unbrined	1 4	2 8	0 9	0 7	3.0	7 3	3.6	0.8	9 7
	Brined	3.6	6 1	1 9	2 0	2 4	1.7	3 5	2 8	10 1
Initial values	Unbrined	0 1		0 0		0 0		0 0	0 0	
	Brined	0 9		0 4		0 3		1 0	0 0	

(3) Two cartons were treated as (2) above, except that commercial washed nitrogen was used in place of carbon dioxide.

(4) Two cartons were placed in an aluminum container and a steady rapid stream of commercial carbon dioxide gas was flushed through it for 30 minutes at slightly above (10 cm. of mercury) atmospheric pressure.

The containers were closed tightly and stored at −10°C. The initial positive internal pressure of 10 cm. of mercury was maintained to allow for any lowering of internal pressure due to cooling of the gas, and also to ensure that, should any slight leakage occur, it would be outward from the containers. After 40 days' storage the containers were opened and one fillet of each fish (brined and unbrined) was removed for examination. The containers were gas treated again as described above, and stored for an additional 58 days, after which the remaining

samples were tested. After both storage periods, the containers had a positive internal pressure which indicated that gas leakage had not been significant.

About one half of each fillet or split fish was used to determine the peroxide value of the fat; the remainder being cooked for a few minutes in boiling water in order to compare its flavour with that of the controls.

It will be seen (table I) that the air-stored samples gave, after storage, peroxide values which were considerably higher than the initial values, especially after 98 days. On the other hand, those which were vacuum-treated and then stored in carbon dioxide or nitrogen gas gave values after 40 and 98 days' storage which were approximately the same, or very little higher, than the initial ones, indicating that no appreciable peroxidation of the fat had occurred. A noticeable increase in peroxide values was found with the samples which had merely been placed in a container and flushed with carbon dioxide, so that sufficient oxygen to cause significant fat oxidation must have remained.

Organoleptic tests were made after both 40- and 98-day storage periods, and the following is a brief resumé of the principal differences observed. The air-stored herring and salmon samples had a marked rancid flavour after 40 days' storage, and this became very pronounced after 98 days. The sole fillets developed a peculiar taste sometimes described as the "salt-fish flavour". While the fillets which were exposed to a vacuum and then treated with carbon dioxide did not have a rancid flavour, they all developed a more or less pronounced undesirable flavour which is difficult to describe and the cause of which is uncertain. Fillets treated with carbon dioxide without previous subjection to a vacuum had a faint rancid flavour after the 98-day storage period, and all possessed the undesirable flavour referred to above. The fillets which were subjected to a vacuum and then treated with nitrogen had a very good flavour and there was no trace of rancidity. Also, the red colour of the salmon flesh was well retained.

GLAZING

The effect of ice glazes formed from water or from solutions of certain antioxidants on the development of rancidity in salmon steaks or fillets has already been described briefly (Tarr 1946b). The results obtained in three typical experiments are recorded herewith.

Six steaks about 1.5 to 2.0 cm. thick (weight 124 to 169 g.), were sawn from a red spring salmon and from a coho salmon, both of which had been dressed, frozen in still air, glazed with ice formed from ordinary water and stored approximately 5 months at about $-28°C$. After weighing them, two steaks from each fish were left untreated, two were glazed with ice formed from distilled water and two with an 0.5% solution of ethyl gallate. Glazing was carried out in a room at about $-18°C$., the steaks being immersed for one minute in solutions at 2 to 3°C. Glazing was found to increase the weight of the steaks from 6.8 to 7.8%. The steaks were wrapped individually in moisture-proof cellophane paper and stored at $-10°C$. Peroxide value determinations (table II) were made at two different storage periods. It will be seen that glazing retarded, but did not prevent, fat peroxidation and that the ethyl gallate glaze was no more advantageous than the water glaze.

TABLE II. Effect of ice glazes formed from water and ethyl gallate solutions on rate of fat oxidation in salmon steaks as shown by peroxide value (initial peroxide values: red spring salmon 0.5; coho salmon 1.2).

Treatment	Red spring salmon		Coho salmon	
After days (no.)	28	79	28	79
Unglazed.................	2.3	17.6	3.2	21.3
Distilled water glaze...................	1.9	6 5	1 9	7 2
0.5% ethyl gallate glaze......	1.6	5 7	1 9	5.8

Ten steaks about 2 cm. thick weighing from 165 to 231 g., cut from a fresh red spring salmon, were wrapped individually in moisture-proof cellophane paper and frozen in still air at −28°C. After one day's storage the frozen steaks were treated as follows:

They were weighed, two were left as controls and two were dipped for 10 seconds in each of the solutions the composition of which is recorded in table III. Glazing was found to increase the weight of the steaks from 5.9 to 6.9%. The glazed steaks were wrapped individually in cellophane paper and stored in wax cartons at −10°C., peroxide values being determined at intervals. The results (table III) show that glazing steaks with ice formed from ordinary water retarded the development of rancidity almost as effectively as did that from 0.5% ethyl gallate solution. On the other hand, glazes formed from 0.5% ascorbic acid solution, or from a similar solution after adjusting it to pH 6.0 with sodium hydroxide, almost entirely prevented fat oxidation.

From a fresh red spring salmon 12 steaks about 1.5 cm. thick (weight 207 to 314 g.) were cut, wrapped and frozen as in the foregoing experiment. Five of the frozen steaks were retained as controls, and five were dipped for 10 seconds in

TABLE III. Effect of ice glazes formed from water or from solutions of ethyl gallate, ascorbic acid or sodium ascorbate (pH 6.0) on rate of fat oxidation in red spring salmon steaks as shown by peroxide value (initial peroxide value zero).

Treatment	After days (no.)	
	41	84
Unglazed......	4 0	8 5
Distilled water glaze	0 7	4 9
0.5% ethyl gallate glaze...	0 6	1 8
0.5% l-ascorbic acid glaze..	0 2	0 2
0.5% l-ascorbic acid glaze (pH 6.0)*....................... ..	0.2	0.2

*An 0.5% solution of l-ascorbic was adjusted to this pH value with 1N NaOH solution immediately prior to use.

TABLE IV.   Effect of ice glazes formed from water and from solutions of ascorbic acid on rate of fat oxidation in frozen red spring salmon steaks as shown by peroxide value (initial value zero).

Treatment	After days (no.)			
	98	101	252	341
Distilled water glaze..	1.7	5.0	20.7	20.4
0.5% 1-ascorbic acid glaze........................	0.5	1.3	3.5	6 8
1.0% 1-ascorbic acid glaze.	0.3	0 6	2.1	4 0

each of the solutions the composition of which is given in table IV, the temperature of the glazing liquids being between 0 and 1°C. The glazing increased the apparent weight of the steaks 5.6 to 8.5%. The steaks were wrapped individually in moisture-proof cellophane, placed in waxed cartons, and stored at −20°C. Peroxide value determinations were made at intervals. The results (table IV) show that the 1-ascorbic acid glazes were much more effective than was the watef glaze, a 1% glaze causing greater protection than a 0.5% one.

DIPPING IN 1-ASCORBIC ACID SOLUTIONS

Fourteen skinned fillets about 12.5 × 7.5 × 2.0 cm. in size were cut from a fresh red spring salmon. Two fillets were dipped for 5 minutes in each of the solutions, the composition of which is given in table V, the temperature of the solutions being maintained between 2 and 5°C. The fillets were drained for about 5 minutes at 0°C., wrapped individually in cellophane paper, air-frozen at −20°C., using a fan, placed in waxed fillet cartons and stored at this temperature. Peroxide value determinations made after two different storage periods (table V) showed that dipping the fillets in 1-ascorbic acid and sodium 1-ascorbate solutions prior to freezing retarded fat oxidation strongly, and to about the same extent.

TABLE V.   Effect of dipping red spring salmon steaks in 1-ascorbic acid or sodium 1-ascorbate solutions on fat oxidation during subsequent storage in the frozen state as shown by peroxide value.

Treatment	After days (no.)	
	45	122
Dipped in distilled water...............	2.6	7.2
Dipped in 0.025% 1-ascorbic acid.....	1.5	2 8
Dipped in 0.50% 1-ascorbic acid.....	0 3	1 6
Dipped in 1.00% 1-ascorbic acid	0 0	0 2
Dipped in 0.25% 1-ascorbic acid pH 6.0*	1 1	3 5
Dipped in 0.50% 1-ascorbic acid pH 6.0*	0 6	1 3
Dipped in 1.00% 1-ascorbic acid pH 6.0*..	0 0	0 2

*Solutions adjusted to pH 6.0 with 1.0 N NaOH solution immediately before use.

TABLE VI. Influence of immersion period on the concentration of 1-ascorbic acid in red spring salmon fillets and on the rate of fat oxidation in frozen stored samples, as shown by peroxide value (initial peroxide value zero).

Immersion period in 1% 1-ascorbic acid solution (minutes)	1-Ascorbic acid content of dipped fillets %	After days (no.)		
		70	120	183
0	0.0025	3 4	10 1	13 1
5	0 0412	0 5	0 9	3 0
10	0 0565	0 3	0 5	2 3 ·
20	0 0778	0 1	0 2	0.6

It will be seen that the rate of fat oxidation was related directly to the concentration of antioxidant in the solutions used for dipping.

From two fresh red spring salmon, twelve skinned fillets about 7.5.×6×2 cm. in size (115 to 125 g. in weight) were cut. Four fillets at a time were immersed in 1% 1-ascorbic acid solution at 1 to 2°C., three different immersion periods being employed. After draining, the 1-ascorbic acid content of one fillet from each treatment was determined (table VI), the remainder being wrapped individually

TABLE VII. Effect of 1-ascorbic acid and related compounds, of derivatives of gallic acid, and of Avenex Concentrate on development of rancidity in minced white spring salmon flesh, as shown by peroxide value.

Compound incorporated in 0.05% concentration	After days (no.)	
	116	199
Control (no compound added) .	11 0	10 3
*1-Ascorbic acid	0 2	0 8
*Sodium 1-ascorbate	0.5	0 8
*d-Iso ascorbic acid	0 2	0 1
*Sodium d-iso ascorbate	0 6	1 1
*5, 6-diacetyl 1-ascorbic acid	0 0	0 5
**Ethyl gallate . .	0 0	1 8
†Gallate E:130#5 .	0 5	2 3
†Isopropyl gallate...	0 5	4 7
†Isobutyl gallate	1 0	6 4
†Dimethyl ethanol ammonium gallate	5 0	5 4
†Diethyl ethanol ammonium gallate	3 1	4 4
†Ammonium gallate	2 4	5 5
†2-Ammonium 2-methyl propanol gallate	5 2	5 9
†Tris (hydroxymethyl) methyl ammonium gallate	2 1	5 7
†Morpholonium gallate . . .	5 7	5 7
††Avenex Concentrate . . .	8 0	10 5

*Hoffmann-LaRoche.
**"Progallin A", Nipa Chemical Co.
†Silmo Chemical Corp.
††Musher Foundation.

in cellophane paper, frozen in air at −20°C. using a fan, and stored at this temperature. The rate of fat oxidation in these fillets was found to depend directly on their 1-ascorbic acid content (table VI).

COMPARISON OF ANTIOXIDANTS

Five-ml. portions of 1% aqueous solutions of the antioxidants listed in table VII were incorporated into minced white spring salmon flesh in the usual manner (Tarr 1947) to give a concentration of 0.05%. Two samples of each treatment were frozen and stored at −20°C., peroxide values being determined after 116 and 199 days' storage. The results (table VII) show that of the various antioxidants studied 5, 6 diacetyl 1-ascorbic acid, 1-ascorbic acid, sodium 1-ascorbate, d-iso ascorbic acid and sodium, d-iso ascorbate all retarded fat oxidation very markedly. Ethyl gallate and gallate E:1305 were also very effective. The remaining gallic acid derivatives studied were all considerably less effective than ethyl gallate. Avenex concentrate proved to be a poor antioxidant.

TABLE VIII. Stability of 1-ascorbic acid in frozen stored white spring salmon flesh.

Days stored (no.)	1-ascorbic acid in the flesh (%)	Peroxide values	
		Untreated flesh	Flesh with 0.05% 1-ascorbic acid added
0	0.049	−	−
4	0 0401	−	−
4	0 0400	−	−
35	0.0371	2.5	0.8
118	0 0346	6 9	0.4
205	0 0294	−	−

STABILITY OF 1-ASCORBIC ACID

1-Ascorbic acid in 0.05% concentration was blended into one portion of minced white spring salmon flesh, the remainder being left untreated. Seven 100-g. portions of each of the treated and untreated fish were wrapped in cellophane in blocks of uniform size and about 1.5 cm. thick. These were frozen in air at −20°C., using a fan, and were stored at this temperature. At intervals samples were removed and partly defrosted, and the 1-ascorbic acid content and peroxide values were determined. The results, given in table VIII, show that the 1-ascorbic acid content dropped sharply at first, probably during the freezing period, and then fell very slowly. Peroxide values increased much more sharply in the untreated samples than in those treated with 1-ascorbic acid.

DISCUSSION

The idea that development of oxidative rancidity in flesh foods might be hindered or prevented by storage in absence of atmospheric oxygen is by no means new. Bronkhuyzen (1927) reported that fat oxidation in fish flesh could be avoided

Erratum: "E:1305" in line 12 above should read "E:130#5"

by packing it in tins in the absence of air before freezing and placing in cold storage. Callow (1933a, b) found that unfrozen pork and bacon could be stored for many weeks at 0 to 1°C. in 96 to 100% carbon dioxide, with little development of rancidity or bacterial growth. In his experiments, storage in nitrogen or hydrogen prevented rancidity development, but occasioned a taint in the flesh. While studying the effect of storage in different concentrations of carbon dioxide on the rate of bacterial spoilage of fish flesh, Weedon and Notevarp (1934) noticed that high concentrations gave noticeable protection against development of rancidity. Kong (1935) studied the effect of storage in vacuum and in carbon dioxide gas on the keeping quality of brined and freshly dried bilis fish (*Stolephorus*). In his tests a reduced pressure of 72.5 cm. of mercury proved most effective, while high carbon dioxide concentrations, although equally good from a preservative point of view, tended to cause a yellow discolouration. Recently, Bucher (1944) has reported that rancidity development in pink salmon fillets can be largely prevented when they are stored in fairly high vacuum. The present experiments have verified certain of the above observations and have extended them to show that storage of frozen fish in a very high partial pressure of carbon dioxide gas prevented fat oxidation, but that it also caused an off flavour in the fish. Storage in nitrogen gas was just as effective in preventing rancidity development and did not cause an undesirable taint. These results, as far as the production of a taint in samples stored in carbon dioxide is concerned, are at variance with those reported by Callow for meat, noted above. The reason for this development of off flavour in gas-stored frozen flesh foods is apparently not known but it may be brought about by traces of impurities in commercial gases used.

Glazing of whole fish with a film of ice before storing them has become a well-established commercial practice, but there seems to be little information available concerning the glazing of individual steaks or fillets. In 1928 Peterson obtained a patent for a process which involved washing pieces of fish or meat with a hypochlorite solution and freezing them rapidly so that they became coated with a thin layer of germicidal ice. Also, certain fishing companies have made a practice of sawing steaks from frozen fish and then glazing them with a thin film of ice in order to protect them until they are used.

The experiments described in this paper have shown that glazing fish steaks or fillets with ice formed from ordinary water decreased the rate of fat oxidation, and that a glaze formed from 0.5 or 1.0% 1-ascorbic acid solution was considerably more effective in this respect. Previous work (Tarr 1947) showed that ethyl gallate, when incorporated into fish flesh in 0.02 to 0.05% concentration, was an excellent antioxidant. In the present experiments a glaze formed from an 0.5% ethyl gallate solution was only about as effective as a water glaze in retarding fat oxidation. This indicates that glazes formed from 1-ascorbic acid solutions may be working as indirect antioxidants by simply removing oxygen from, and preventing its access to, treated flesh.

Individual fillet or steak glazing does not seem to be a very desirable process from a commercial standpoint, for it causes an apparent increase in weight of treated samples, the glaze may thaw and leave a quantity of free liquid in the

package, and there is a tendency for the glaze to soak into the flesh on defrosting. Antioxidant glazes for whole fish are now being investigated.

It has been found that 1-ascorbic acid can readily be incorporated into fish flesh by dipping fillets or steaks into solutions of either the acid itself or its sodium salt (pH 6.0), and that both procedures appear to be equally effective. Within tested limits of 0.025 to 0.078%, 1-ascorbic acid proved considerably more effective as an antioxidant for fish flesh when used in the higher than in the lower concentrations. When 1-ascorbic acid was incorporated into fish flesh which was promptly frozen and stored, its concentration fell rapidly during the first day of storage and subsequently much more slowly. This indicates that there is probably a marked oxidation of 1-ascorbic acid in unfrozen fish flesh, and that in frozen flesh the oxidation is relatively slow.

SUMMARY

Oxidation of flesh fats of frozen fish and development of attendant rancid or related off flavours was prevented by storing it in very high partial pressures (total pressure 10 cm. of mercury above atmospheric) of carbon dioxide or nitrogen gases. Storage in carbon dioxide caused a noticeable off flavour in all samples, which was not observed in those stored in nitrogen.

Glazing individual frozen salmon steaks or fillets with ice formed from ordinary water retarded the rate of fat oxidation when the samples were stored. A glaze formed from 0.5 or 1.0% 1-ascorbic acid or sodium 1-ascorbate (pH 6.0) solutions was considerably more effective in retarding oxidation of fat than an ordinary water glaze, while a glaze formed from 0.5% ethyl gallate solution was only about as effective as a water glaze.

1-Ascorbic acid was readily introduced into fish fillets or steaks by dipping them in 0.5 or 1.0% solutions either of the free acid, or of its sodium salt (pH 6.0). Both procedures gave about the same degree of protection against development of rancidity in frozen stored samples. High concentrations of 1-ascorbic acid in frozen fish flesh (e.g. 0.078%) were considerably more effective than low concentrations (e.g. 0.025%) in retarding fat oxidation.

d-Iso ascorbic acid, sodium d-iso ascorbate, and 5, 6 diacetyl 1-ascorbic acid gave, on a molecular basis, about the same degree of protection against oxidation of cold stored fish flesh fat as did 1-ascorbic acid or its sodium salt. All these compounds were as effective, or more effective, than ethyl gallate in the same per cent concentration. Other derivatives of gallic acid were less effective than ethyl gallate, while Avenex Concentrate offered but little protection.

ACKNOWLEDGEMENTS

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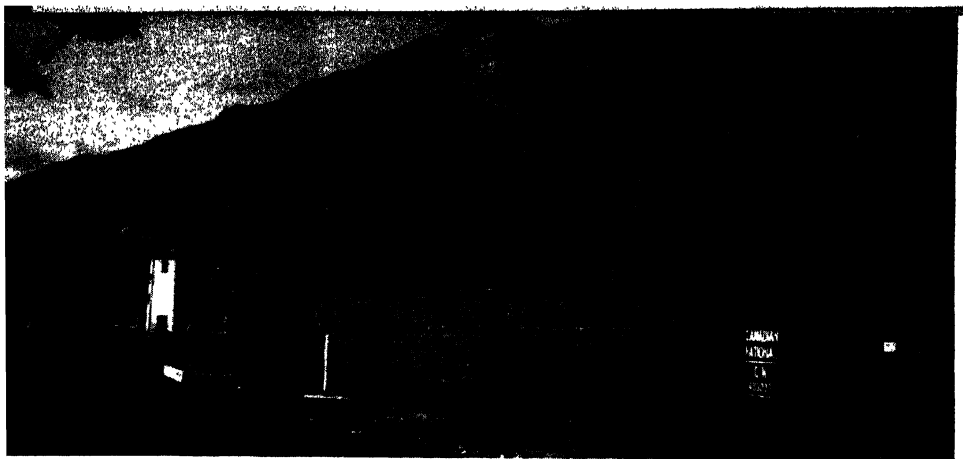
Fig. 1. Test train, consisting of two refrigerator cars, tourist car, and freight car for carrying supplies, stopped in foothills of Canadian Rockies.

Refrigerator Car Road Tests with New Cooling Mixtures

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I N these trials the addition of ammonium nitrate to the sodium chloride-ice mixtures lowered the bunker temperatures approximately 7 F, resulting in temperature reductions of 2.5 to 5.5 F within the cars. Analysis of the results shows that the size of ice used in the

A previous paper, published in the March 1948 issue of REFRIGERATING ENGINEERING, demonstrated that, in a sectional model of an overhead brine bunker type of railway refrigerator car, it was possible to lower the bunker temperatures of regular sodium chloride-ice mixtures approximately 10 F by the addition of small percentages of ammonium nitrate to these mixtures. This paper also pointed out that the corresponding temperature reductions within the car may be in the order of 6 to 8 F, resulting in a significant improvement over present conditions. To assess these ammonium nitrate-sodium chloride-ice cooling mixtures in actual practice, two railway refrigerator car road tests were undertaken during the summer of 1947 and are reported in this paper.

bunkers had an appreciable effect on the resulting temperatures, and indicates that if finely crushed ice had been used at all icing stations, temperature lowering would have been of the magnitude expected from the laboratory tests in the model car, reported in the March 1948 issue of REFRIGERATING ENGINEERING.

Test Conditions

Each test unit consisted of two refrigerator cars, a tourist car for office use and accommodation of per-

sonnel, and a freight car to carry ammonium nitrate and auxiliary supplies. The refrigerator cars were of the overhead brine bunker type employing eight bunkers per car, four along each side of the roof. Boxed frozen fish was the commodity carried.

The first test, made over the Canadian Pacific Railways from New Westminster, B.C., to eastern Canada, used two cars of the same design (C.P. 281818 and C. P. 281200, hereafter referred to as Cars F. C. and F. A., indicating First Test, Control Car; and First Test, Ammonium Nitrate Car, respectively). Both cars employed 3½ in. of hairfelt insulation on the side and end walls, 4½ in. of hairfelt insulation on the floor and ceiling, and had "wet" side wall ducts—i.e., the brine overflow from the bunkers might run down any portion of the side wall ducts. Car F.C. was iced with 25 lb sodium chloride per 100 lb ice; car F.A. received the same quantities of ice and sodium chloride but, in

Fig. 2. Thermocouple and resistance thermometer leads tied to tops of test cars from which location they are returned to tourist car for measurement.

addition, 12½ lb ammonium nitrate per 100 lb ice. Since car F.C. was billed to Toronto, Ontario, and the other to Montreal, Quebec, the comparison of resulting temperatures ended at Toronto.

The second test, conducted over the Canadian National Railways from Prince Rupert, B.C., to Montreal, Quebec, also employed two refrigerator cars (C.N. 210219 and C.N. 210218, hereafter referred to as cars S.C. and S.A., indicating Second Test, Control Car; and Second Test, Ammonium Nitrate Car, respectively). These cars were of the same design, employing 3½ in. hairfelt insulation on the side and end walls, 4½ in. hairfelt insulation on the floor and ceiling, and had "dry" side wall ducts—i.e., the brine overflow from the bunkers was restricted to approximately 1/10 of the side wall area. Car S.C. was iced with 30 lb sodium chloride per 100 lb ice; car S.A. received 15 lb am-

monium nitrate per 100 lb ice in addition to the amount of sodium chloride used in the control car.

The refrigerator cars were iced approximately once daily while in transit.

Temperature Measuring Equipment

Temperature sensitive elements were placed in each of the refrigerator cars and the lead wires brought back to the tourist car where the temperature measurements were taken. Since the shipments were moving in an easterly direction, these elements were mainly con-

Fig. 3. View showing how temperature measuring lead wires were passed through refrigerator car door.

centrated in the southeast quarter of the loadings. Because of the prolonged exposure of the sun on this section of the car, it was thought that this portion of the loading should be at the highest temperature.

Eight resistance thermometers were employed to obtain the air temperature in the car; two of these were placed beneath the floor racks and six were distributed in the air space above the load. These are subsequently referred to as *air temperatures*.

The remaining temperature measurements were made with thermocouples. Three of these were frozen in the product prior to loading and represented one box in a triple corner, a second box in the periphery of the load, and a third box in the interior. These are subsequently referred to as *product temperatures*.

Nine thermocouples were distributed throughout the loading between the boxes. Since these thermocouples are affected by both the product and air, they are referred to throughout as *product-air temperatures*.

Thermocouples were placed in brine bunkers of each car to obtain the temperature of the cooling mixtures. On the first test two such elements were used in each car whereas the second test employed four per car. These elements were in direct contact with the cooling medium and were supported approximately 1 in. above the bottom surface of the tanks. The bunker measurements for each car were averaged and are subsequently referred to as *bunker temperatures*.

More temperature-sensitive elements would have

Table 1. Comparison of Temperatures (averages) and Ice Consumption

Item	Test No. 1			Test No. 2		
	Temp., F, Car F.C.*	Temp., F, Car F.A.*	Temp., °F, difference	Temp., F, Car S.C.*	Temp., F, Car S.A.*	Temp., °F, difference
Outside air (shade)	64.7	64.7	—	67.0	67.0	—
Bunkers	—2.8	—10.1	7.3	—3.3	—10.3	7.0
Car air	11.4	8.0	3.4	12.4	7.0	5.4
Product-air	10.2	7.0	3.2	14.0	9.9	4.1
Product, loading	4.0	4.0	—	15.2	11.6	(3.6)
Product, transit	8.1	5.6	2.5	14.0	9.8	4.2
Product, destination	10.0	7.2	2.8	14.6	10.9	3.7
Ice consumed, transit	16,300 lb	18,900 lb	2600 lb	16,900 lb	19,500 lb	2600 lb
Time, transit	172 hr	172 hr	—	185 hr	185 hr	—

* Car F.C.—25 lb NaCl/100 lb ice   Car S.C.—30 lb NaCl/100 lb ice
Car F.A.—25 lb NaCl + 12.5 lb NH₄NO₃/100 lb ice   Car S.A.—30 lb NaCl + 15 lb NH₄NO₃/100 lb ice

been desirable but the numbers used here were dictated by the quantity which could be conveniently used.

Precooling

A record of car air temperature during the precooling period was obtained by placing a calibrated recording thermograph in a central position on the floor rack of each car. On both tests, the car doors and hatchways were open for two days prior to precooling, to ensure that the interior of the car and the car structure would be at the ambient temperature.

In the first test, both cars were precooled by icing to capacity with their respective test mixtures, and re-icing 27 hr later. Loading of produce was started 47 hr after the initial icing. The car precooled with 25 lb sodium chloride per 100 lb ice reached a minimum of 18 F at the end of the first 24 hr, after which continued cooling resulted in no further temperature depression. The same results were obtained with the car employing ammonium nitrate, although its minimum temperature was 15 F. These data show that the addition of ammonium nitrate is not justified for precooling purposes. Temperatures as much as 8 F lower[1] would have been expected had the cars been in motion.

The cars of the second test were precooled for 18 hr with 30 lb sodium chloride per 100 lb ice. At the end of this period, both cars registered internal temperatures of 18 F. However, the precooling curves indicated that additional cooling might have resulted in the temperatures being lowered another 1 to 2 F.

Both tests show that, with the type of car used here, the maximum from precooling is achieved in 24 hr.
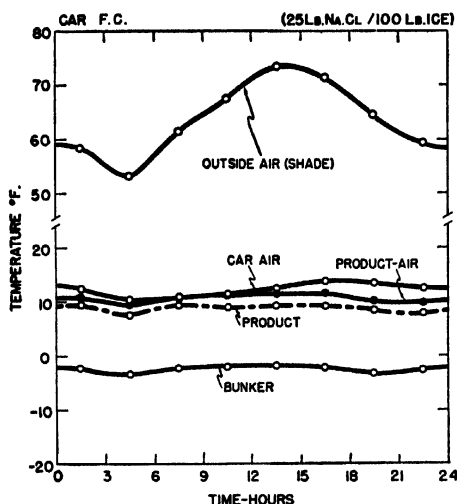
Temperature Measurements

A complete set of temperature measurements was recorded once every 3 to 4 hr during the seven days of each test. In general, the temperatures showed some irregularities and a tendency to increase with time. To analyze these results, the observations were grouped into the differences attributable to within and between days, and are shown in Figure 4 for the first test, and in Figure 5 for the second.

Before considering the test results, it is important to note the difference in product loading temperatures. On the first test the product at loading was approximately 4 F, a temperature below that attained at any subsequent time in the refrigerator cars. On the second test the product was loaded at 11 to 15 F, temperatures equal to or above those attained at any subsequent

time in the cars.

Temperature Variations within Days—To determine the variations within days, the results were grouped by three-hour intervals, starting at midnight, the averages being taken over all days in any one test. The results appear in the top halves of Figures 4 and 5. It is evident that the outside air temperature attained a maximum at about 13:30 hr, with a minimum near 4:30 hr. No distinct peaks were observed in the temperatures taken within the cars, although the charts suggest a maximum in the car air between 15:00 and 18:00 hr on both tests, and a minimum in the vicinity of 6:00 hr on the first test and 7:30 hr on the second. It is
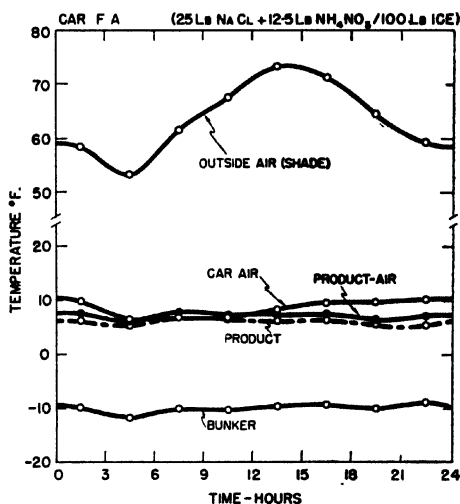
Fig. 4. Temperatures for hours in day and between days for first test.

also obvious that the bunker temperatures were substantially lower in the cars iced with ammonium nitrate. This lowering in temperature is reflected to a lesser extent in the air, product, and product-air temperatures.

Temperature Variations between Days—To obtain an indication of the changes between days, an average temperature was computed for each day. These are plotted in the lower halves of Figures 4 and 5. The outside shade temperatures reflect the meteorological conditions encountered. For the first test the temperature of the air in the car was slightly above that of the product-air and this, in turn, was slightly higher than that of the product; however, during the second test, when the product was loaded at a higher temperature

TEMPERATURES WITHIN DAYS
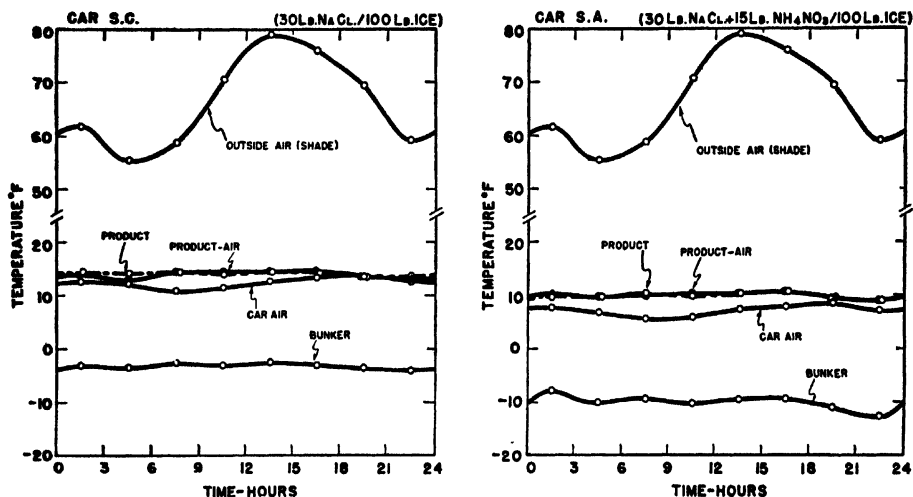
TEMPERATURES BETWEEN DAYS
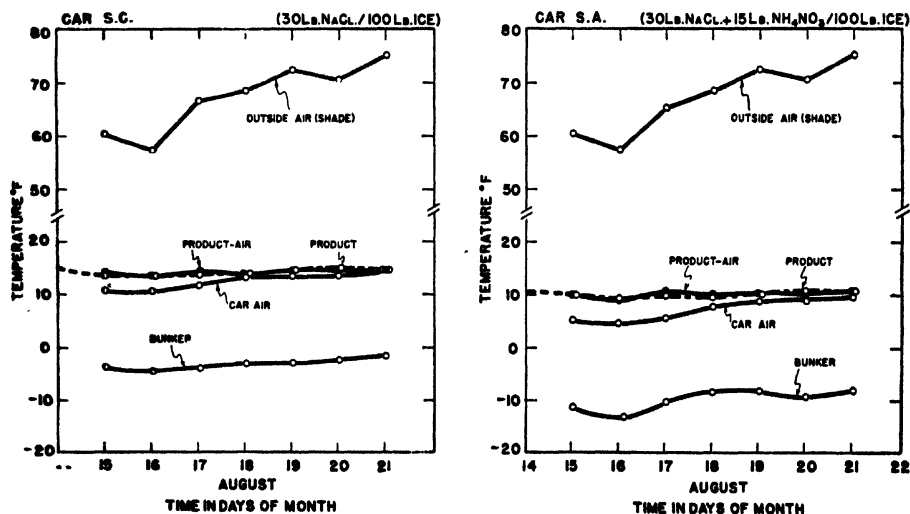
Fig. 5. Temperatures for hours in day and between days for second test.
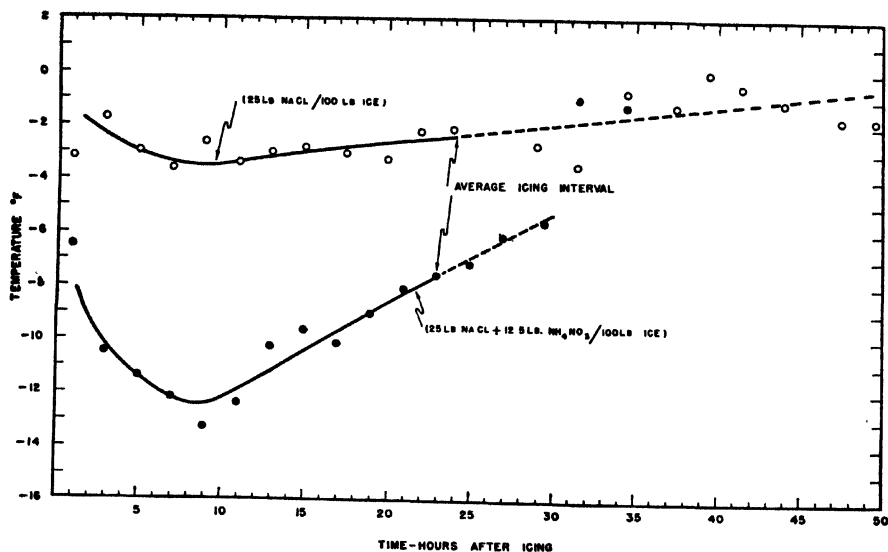
Fig. 6A (above). Bunker temperature versus time after icing for Test No. 1.
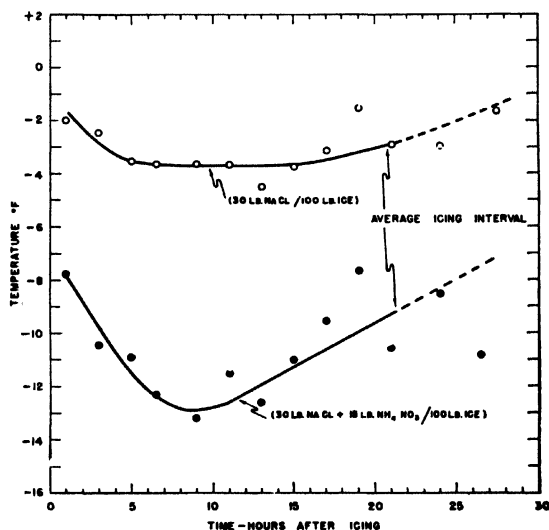
Fig. 6B (right). Bunker temperature versus time after icing for Test No. 2.

the reverse of these conditions existed. Otherwise these temperatures are quite regular and show only a slight tendency to increase with time. The most notable differences are in the bunker temperatures. In the first test these reached a maximum after three or four days in transit and in the second test they tended to increase throughout. This is attributed to the size of ice used at midcontinent icing stations. It is evident that the bunker temperatures in the cars receiving ammonium nitrate were more variable than those in the cars receiving sodium chloride alone.

Bunker Temperatures in Relation to Icing Time and Ice Size—In view of the variations in bunker temperature noted in the previous section, the results were analyzed to determine the relation between bunker

temperatures and time of icing. In general, the cars
were iced once every 24 hr, but actually times between
icing varied from as little as 10 hr to as much as 49 hr.
The bunker temperatures were therefore averaged by
two-hour intervals and plotted against the time from
the last icing. The results for both tests appear in
Figs. 6A and 6B. These charts show that in the cars re-
ceiving sodium chloride only, the minimum temperature
was attained 8 to 10 hr after icing, and subsequently the
temperature increased in a linear fashion at the rate
of about 1 F in 10 hr. On the other hand, the cars
receiving ammonium nitrate in addition to sodium
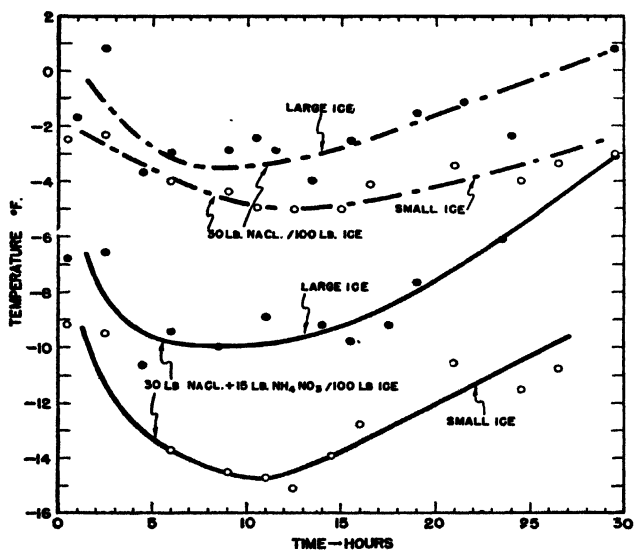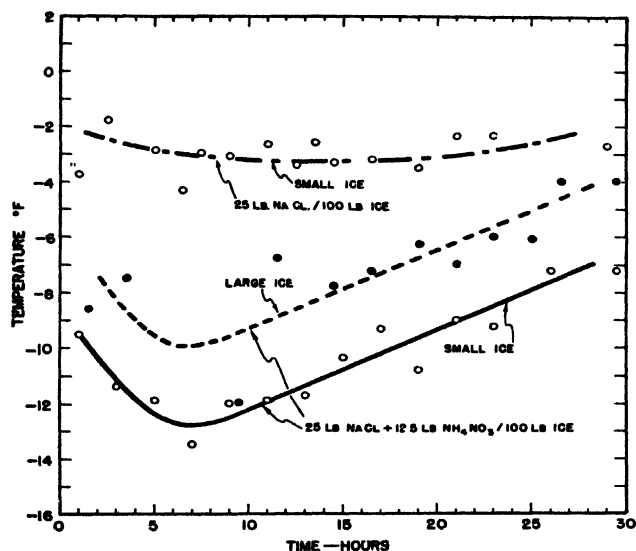
Fig. 7. Effect of ice size upon bunker temperature
(bunker temperature versus time after icing). Test No. 1
at top and Test No. 2 below.

chloride showed a minimum about 9 hr after icing, and subsequently the temperature increased at a rate of 3.5 F in 10 hr.

Further analyses of these data were performed in essentially the same manner, but separating the stations at which large and small ice was used (considered to be large when half or more of the ice would require at least a 6 in. sq mesh if sifted, and small ice when all pieces would pass through a 2 in. sq mesh). The results for both tests appear in Figure 7. In the first test, large ice was obtained at only a few stations and as the car receiving sodium chloride only did not require ice at one of these, the results were too limited to justify plotting. In the car receiving ammonium nitrate, however, it is evident that, while the results with large ice were limited, the bunker temperatures obtained were some 3 F higher than those obtained under comparable conditions with small ice. The second test reflected the same behavior. In the car receiving sodium chloride only, the small ice yielded bunker temperatures at least 2 F lower; in the car receiving ammonium nitrate the temperature difference between large and small ice size attained a level of 5 F.

Discussion

In these operating tests the addition of ammonium nitrate to regular sodium chloride-ice mixtures lowered the average bunker temperatures about 7 F below the comparable control. This is somewhat less than the 10 F lowering observed for ammonium nitrate additions in laboratory tests[1]. Most of this difference can be attributed to the effect of ice size: the use of large sized ice during several days of the shipping trial caused a 3 to 5 F increase in bunker temperatures (Fig. 7) during that period. When the bunker temperature is plotted against time from last icing (Figs. 6 and 7), the form of the curve suggests rapid solution of the ammonium nitrate to produce minimum temperature followed by its dilution or loss (through overflow), resulting in a rapid temperature rise. This possibility is now under investigation. The results also show the necessity for re-icing at least once every 24 hr if minimum temperatures are to be maintained.

The 7 F reduction in bunker temperature lowered the temperature of the car air 3.4 F in the first test, and 5.4 F in the second. A higher proportion of sodium chloride and ammonium nitrate was used in the second test, but comparisons between trials were vitiated by the wide difference in product temperatures at the time of loading (Table 1).

The cars receiving ammonium nitrate consumed 16.0 and 15.4% more ice than the corresponding control cars. If the overall temperature difference between the car and ambient air is proportional to the ice consumption, the cars receiving ammonium nitrate should have been 7 to 8 F lower than the control cars. This ideal relation can hardly be expected and the high product temperatures used in the second trial doubtless prevented the attainment of minimum car temperatures.

The increased cost of ammonium nitrate additions will be determined by the cost of this component plus the additional quantities of salt and ice required. These costs will vary depending on the place, time, etc. Estimates based on the present trials indicate that the use

of ammonium nitrate would increase the strict refrigeration costs up to 50%, but this would be a much smaller percentage of the cost of freight and refrigeration.

Acknowledgments

Reference

1. Carbert, J. M., and W. H. Cook; *Refrig. Eng.*, vol 55, p 251, 1948

THE EFFECT OF METHOD OF FREEZING, TYPE OF PACK AND STORAGE ON ASPARAGUS TISSUE

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THE EFFECT OF METHOD OF FREEZING, TYPE OF PACK AND STORAGE ON ASPARAGUS TISSUE[1]

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In conjunction with the determination of rates of freezing for several commercially packed vegetables and fruits (2) this investigation was undertaken with a view to ascertaining the extent and location of damage to the tissues by different methods of freezing, packing and periods of storage.

MATERIALS AND METHODS

Unpacked and packed asparagus was frozen by different methods which involved varied rates of freezing. For complete freezing and hardening the freezing period ranged from under 2 minutes to practically 12 hours. The first examination of all products was made approximately 24 hours after placement in the freezing room. At this examination, made in a cold room of + 10° F. to + 20° F. to prevent thawing of the material, freehand and, when possible, microtome sections were cut and examined microscopically. Duplicates were fixed in the chilled fixing solutions. Either a modification of Bouin's solution or form-acetic alcohol was used. The material was then dehydrated in an alcohol series, stained and mounted. A third lot was fixed in the cold room and pumped while thawing in the laboratory. This latter material was dehydrated in an N-Butyl alcohol series, embedded in paraffin and later stained variously, haematoxylin and crystal violet proving the best.

All ice crystals dissolved in the fixing solutions but the patterns of tissue rupture were fixed. Contiguous sections from the same piece of frozen tissue examined while frozen or fixed and mounted in the cold room or sectioned from embedded material indicated the pattern similarity.

Additional material was obtained from beans, strawberries and peas, but frozen strawberries fractured so readily during the sectioning operation that sections for mounting were difficult to obtain, and the hard akenes in the embedded material caused shredding.

Two years' work with Washington asparagus included:

Brine pack, static at −50°, −20° and 0° F.,
Dry pack, contact at −50° and −20° F.,
Dry and brine packs, airblast on product at −50° F., −20° F. and 0° F., packaged after freezing,
Dry and brine packs, immersion of product at −50° F. and at 0° F., packaged after centrifuging the frozen material, and
Dry and brine packs, airblast at −20° F. and at 0° F. on the packaged product.

For static freezing, cylindrical, heavily-waxed, pint cartons were filled with the prepared asparagus spears, covered with 2 per cent NaCl solution then frozen in still air. Contact frozen cartons were of dimensions $5\frac{1}{4}'' \times 5\frac{1}{4}'' \times 2\frac{1}{4}''$ and were placed between metal plates between the expansion coils. Asparagus frozen on agateware trays directly in front of

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[1] Contribution No. 673 from the Division of Horticulture, Experimental Farms Service, Ottawa.
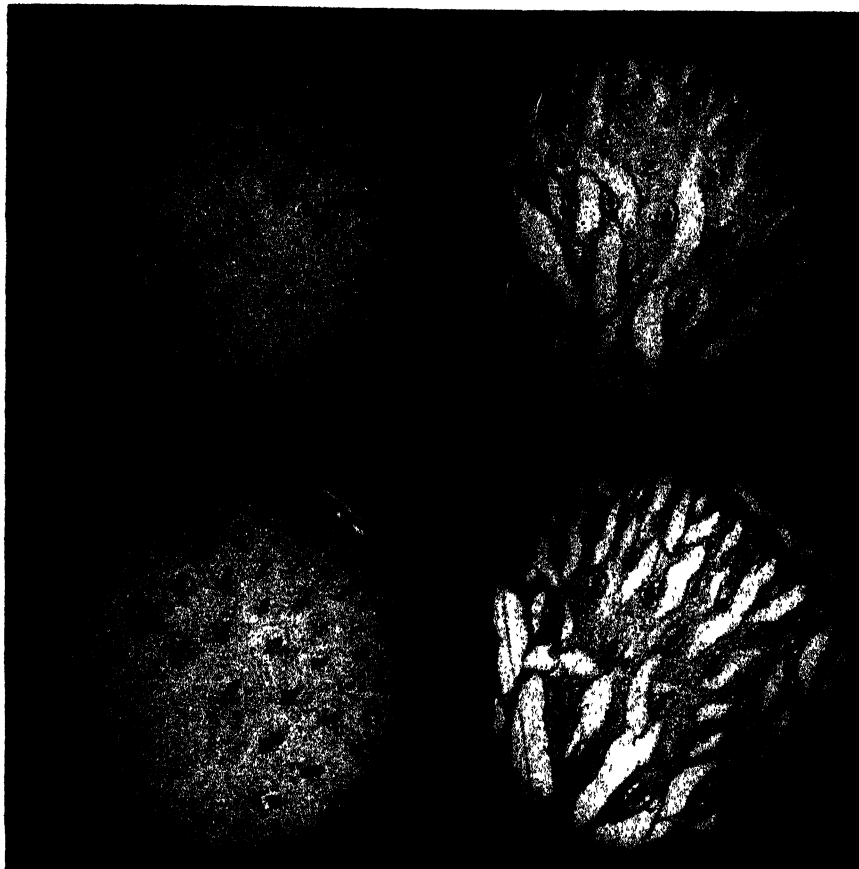
PLATE I: FIGURE 1 (upper left). Cross section of fresh asparagus, 10X
FIGURE 2 (lower left). Cooked unfrozen asparagus, 10X.
FIGURE 3 (upper right). Brine-packed asparagus, frozen static at −50° F. Stored 24 hr. 10X.
FIGURE 4 (lower right). Brine-packed asparagus, frozen static at 0° F. Stored 24 hr. 10X.

fans, then packaged either dry or in two per cent brine, was the method of freezing by airblast on the product. For immersion, the prepared material was placed in wire baskets, the baskets immersed in the freezing solution, centrifuged after freezing, then packaged either dry or in brine. Airblast of 450 l.f.p.m. on the pack is self-explanatory.

RESULTS

Static Freezing

The static brine packs were 60° F. at the time of placement in the freezing rooms. To reach the freezing room temperature the samples frozen at −20° F. required 485 minutes, and those at 0° F., 765 minutes. Figures were not obtained for the −50° F. static freeze but observations and calculations indicate that it should be about 325 minutes.

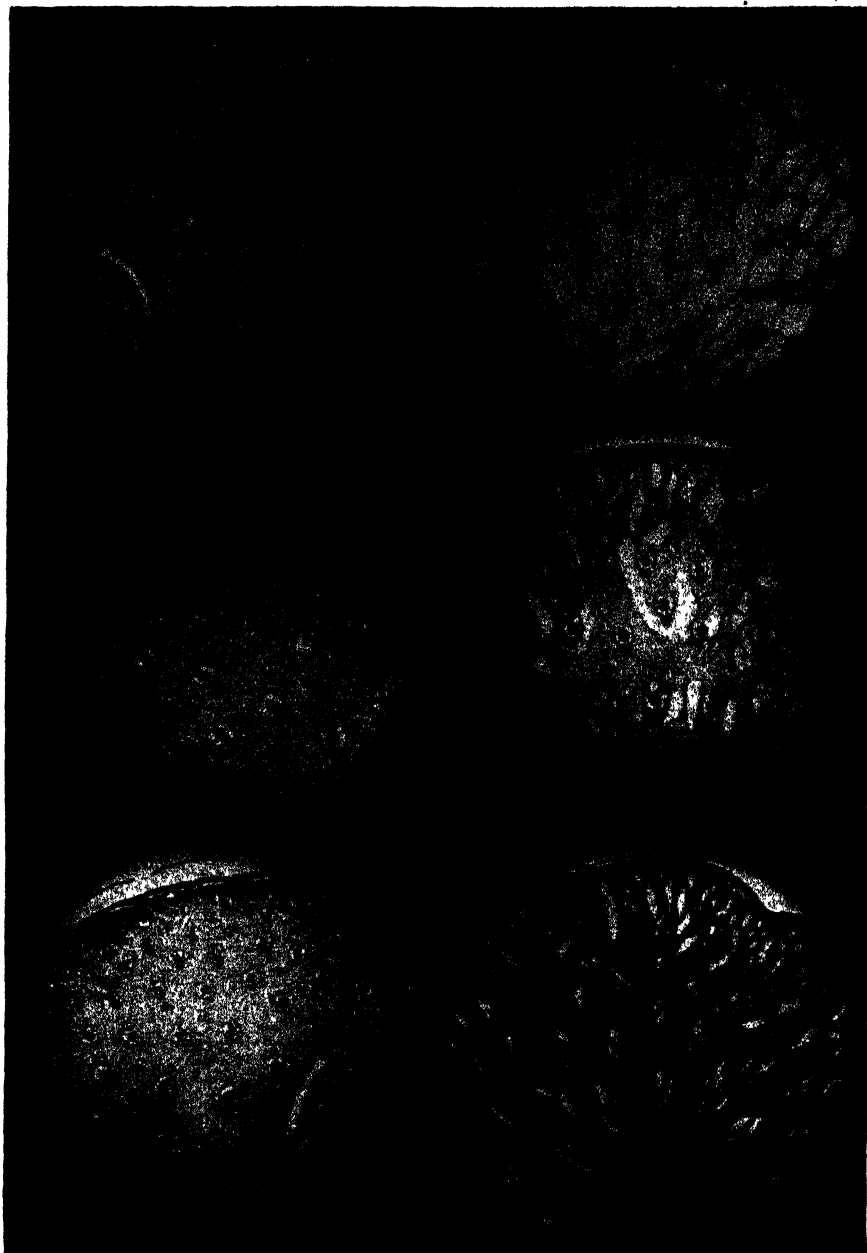PLATE II: FIGURE 1 (upper left).   Asparagus frozen at 0° F. by airblast of 450 l.f.p.m.
                                                    on the unpacked product.   Stored 24 hr.   10X.
              FIGURE 2 (centre left).   Asparagus frozen at −20° F. by airblast of 450 l.f.p.m.
                                                    on the unpacked product.   Stored 24 hr.   10X.
              FIGURE 3 (lower left).   Asparagus frozen at −50° F. by airblast of 450 l.f.p.m.
                                                    on the unpacked product.   Stored 24 hr.   10X.

PLATE II: FIGURE 4 (upper right).   Brine-packed asparagus frozen at 0° F. by airblast
(continued)   of 450 l.f.p.m. on the pack. Stored 24 hr. 10X.
            FIGURE 5 (centre right).   Dry-packed asparagus frozen at 0° F. by airblast
                                                 of 450 l.f.p.m. on the pack. Stored 24 hr. 10X.
            FIGURE 6 (lower right).   Brine-packed asparagus frozen at −20° F. by airblast
                                                 of 450 l.f.p.m. on the pack. Stored 24 hr. 10X.

At the 24-hour examination the tissue of the statically frozen brine-packed asparagus was severely ruptured (Plate I, Figures 3 and 4). The tears in the tissue extended one to several centimeters longitudinally and had a radial diameter of up to one centimeter with a smaller tangential diameter. The tears were smaller, but more numerous near the circumference of the stalk where the bundles were also more numerous. These tears were packed with ice crystals, the crystals in the asparagus frozen at the highest temperature being the largest. However, in these three brine-packed, statically frozen lots it was difficult to obtain microtome sections of the frozen product; the ice crystals "popped" out of lacunae on cutting, and the sections would collapse during transference to the fixing solutions. The large lacunae appeared cross-barred by the regular ice crystal cleavage planes, but the crystals were packed irregularly in the smaller lacunae. The entire stalks of these statically frozen samples were encased in a hull of ice. On sectioning the frozen mass, this hull broke off in flakes and frequently removed areas of epidermis. Just beneath the surface, the larger diameters of the small lacunae were tangential and this assisted in tearing the epidermis during sectioning. The larger lacunae were filled with roughly cubical ice crystals. In the centre of each ice crystal there was one to several small air or gas bubbles. When several were present they were not grouped but in a straight line. There appeared to be a layer of ice-crystals inside the cell close to the cell wall. These could be seen clearly in the product frozen at −50° F., but in the material frozen at higher temperatures it was not possible to cut sections thin enough to determine whether ice formation occurred in the cells or the intercellular spaces.

There were variations in the cavitation pattern, that is, certain samples frozen at −50° F. were very similar to samples frozen at higher temperatures and occasionally one of these latter samples exhibited less cavitation than others frozen more rapidly. Some of these differences were probably due to the physiology of the individual stalk, for it was noted that tips with highly metabolic cells still in the actively dividing or expanding stage, and thus full of cytoplasm with few vacuoles, were more severely torn with flattened and crushed cells. This was the case no matter what the temperature of freezing.

With storage there was, in these cases, an increase of tissue rupture. This increase in the tissue rupture was not so conspicuous in the brine-packed statically frozen material since the lacunae formed by the ice crystals were very large at the first examination.

Contact Freezing

By calculation contact freezing of the dry pack at −50° F. required 170 minutes. By this method of freezing the tissue was not ruptured extensively (Plate III, Figure 3). In the more developed part of the stalk

the tears generally involved the fibrovascular bundles with the conducting tissue torn and the phloem crushed. In rather curved tears between or around bundles the largest was approximately 5 millimeters in radial and 2 millimeters in tangential diameter. At the sides of such cavities the cells were crushed and flattened. The ice crystals here were small. The undamaged cells were fully distended, some with many small ice crystals. In certain cases, the intercellular spaces contained ice crystals; in others, there were none at these locations. Here, as in immersion, the cell sap in general was not withdrawn from cell to intercellular space and therefore was frozen *in situ*. With storage, tearing increased slightly. Contact at −20° F. required 285 minutes for freezing and hardening. Cavitation in these samples (Plate III, Figure 4) was practically as extensive as in the brine pack static freeze at −20° F. requiring 485 minutes.

Freezing by Airblast on the Product

With airblast on the product those samples frozen at −50° F., −20° F. and 0° F. required 11, 18 and 32 minutes, respectively. The period for −20° F. freezing was obtained by observation; the others by calculation. Tearing of the tissue with these methods of freezing was slight (Plate II, Figures 1, 2 and 3). The ice crystals were small and were for the most part in the cells but the cavities were filled. Tearing, in general, involved the fibrovascular bundles but some few cavities appeared between bundles.

Dry-packed asparagus frozen by this method showed an increase of cavitation with storage of 8 to 10 months. This was more noticeable with −50° F. and 0° F. freezing, but those samples frozen at −50° F. were stored at the same temperature for 3 months and at 0° F. for the remainder of the period. The samples packed in brine after freezing were considerably more ruptured by the ice-filled cavities than the dry-packed products and the ice crystals in the cavities were also larger.

Freezing by Immersion

Immersion freezing, the fastest freezing employed, caused the least rupture of the tissues. The freezing period for either −50° F. or 0° F. was not obtained, but at −25° F. the interval required was under 2 minutes. At the first examination asparagus sections from stalks frozen by immersion at −50° F. showed only an occasional broken xylem element in the fibrovascular bundles near the periphery of the stalk (Plate III, Figure 1). The ice crystals were very fine and were definitely within the cells. With immersion at 0° F. a small degree of tissue rupture occurred at the fibrovascular bundles and occasionally between the bundles (Plate III, Figure 2). Small radial tears were closely massed at the periphery.

With storage for 6 months at 0° F. there was practically no increase in tissue rupture of the dry-packed product, but ice-filled lacunae appeared in the centre of the stalks of the brine-packed material. An increase of tissue rupture occurred during storage in both dry and brine packs frozen at −50° F., the rupture in the latter being more extensive than with brine pack at 0° F. Again, this may be due in part to the change of temperature from −50° F. to 0° F. after 3 months of storage.
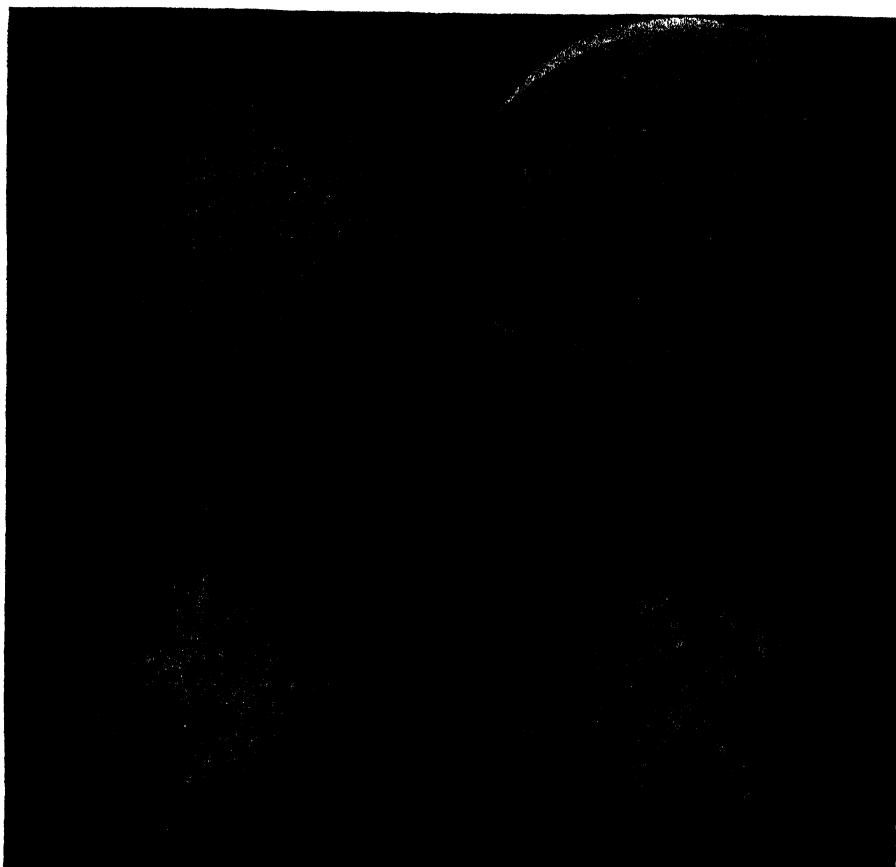
PLATE III: FIGURE 1 (upper left).   Asparagus frozen by immersion at −50°F. Stored 24 hr. 10X.

   FIGURE 2 (lower left).   Asparagus frozen by immersion at 0°F. Stored 24 hr. 10X.

   FIGURE 3 (upper right).   Dry-packed asparagus frozen at −50°F. by contact of the package with freezer plates.

   FIGURE 4 (lower right).   Dry-packed asparagus frozen at −20°F. by contact of the package with freezer plates.

Freezing by Airblast on the Packed Product

The freezing and hardening periods for airblast on the brine-packed samples were 165 minutes at −20°F. and 255 minutes for 0°F., while airblast on dry-packed samples at 0°F. took 330 minutes. Although the brine pack (Plate II, Figure 4) was frozen more rapidly than the dry pack (Plate II, Figure 5) there was more extensive rupture of the asparagus tissue and the cavity pattern was quite similar to freezing a brine pack static at 0°F. Tissue rupture of the brine-packed material frozen at −20°F. was quite extensive, involving the vascular bundles and the periphery of the stalk (Plate II, Figure 6). Ice crystals in the −20°F. sample were smaller than in those frozen at 0°F. There was little to no increase of cavitation in these samples with storage at the temperature employed for freezing.

DISCUSSION

There is, of course, no tissue rupture in the fresh product (Plate I, Figure 1). A very few small tears occurred in the fresh cooked material, but bundles were not involved (Plate I, Figure 2). In all frozen asparagus, bundles were more or less involved indicating that breakdown or tearing of tissue at bundles is due to ice crystal formation. This involvement of the bundles whether or not parenchyma tissue is also ruptured has been reported by Woodroof (3). Lee, Gortner and Whitcombe (1) stated that "the slower the rate of freezing, the larger the ice crystals; but in the corresponding thawed samples these differences disappeared, and damage was not apparent." Their photographs of frozen peas and beans show increased cavitation with extended freezing periods and the cavitation is not apparent in the thawed product.

As already stated, the asparagus frozen at this laboratory was examined while frozen and slides were made from material that was fixed while in the frozen state. The material was not allowed to thaw before fixation. However, samples were also obtained from lots which were fixed after cooking the frozen asparagus. The slides—and of course the photomicrographs therefrom—all show the same cavitation as in the frozen product. It would appear that in the thawing the cavities present in the frozen material are obscured by the cytoplasm, water and those organic fragments loosened during cutting and therefore, though not apparent, the cavities are still present.

The larger cavities in slow-frozen brine-packed asparagus, which may extend radially across 125 or more cells and tangentially across 50 cells, are fairly easy to see with the naked eye; but in the fast-frozen product they may be less than a dozen cells in length and four to five, sometimes only one or two, cells in width. Such cavities are not visible to the unaided eye, or they may be occluded by free cytoplasm or cell fragments flooding them during thawing. Unless observed by magnification of the stained thin sections the tissue of airblast frozen asparagus, for example, does not appear to be torn, crushed or disrupted.

Yet the cavitation of slowly frozen or brine-packed products has little effect on the texture to the consumer. This is indicated by taste panel results on beans. The average texture value of 56 samples of cooked brine packed beans was 86.5, and of dry-packed, 86. Seven methods of freezing were included in these tests, but by all methods there were both brine- and dry-packed samples and tissue rupture in the brine pack is relatively greater than in the dry pack frozen by the same method.

When ice-filled cavities extend for as much as 5 centimeters vertically in a stalk of slow-frozen brine-packed asparagus, it is reasonable to deduce that more extensive leaching occurs during the thawing and cooking of the product. However, the average ascorbic acid content of 128 dry packs of beans was 16.9 mg. and of the brine packs 17.2 mg. per 100 gm. of frozen solids. In the cooked products, with approximately half the number of samples, the solids in the dry and brine packs contained respectively 9.7 and 7.34 mg. per 100 gm. The cooking liquids of these contained 3.8 and 3.96 mg./100 gm. based on the solids content. Therefore, although the cooked dry-packed material which had smaller ice-filled cavities in

the frozen product retained more ascorbic acid than the brine-packed, and although the ascorbic acid in the cooking liquid was practically the same, it may be argued that the greater losses in the brine pack were not due to more leaching but to necessarily longer exposure to heat for thawing and cooking. No packs were thawed at room or refrigerator temperature before cooking. On the other hand the cooked values of dry-packed beans frozen by airblast on the pack at $-20°$ F. and by static at $0°$ F., and therefore with considerable differences in cavitation, averaged respectively 10.5 and 8.2 mg./100 gm. ascorbic acid in the solids. These samples were processed from one lot of beans thus reducing the fresh factor difference. The ascorbic acid contents of the cooking liquids were 2.1 and 3.0. The values of the uncooked frozen products were 12.8 and 12.9. Since the difference between these two lots was a difference in freezing time and consequent difference in tissue rupture only, it is reasonable to conclude that in this case the greater vitamin retention in the cooked solids is due to less tissue rupture in the faster frozen sample.

Although Woodroof et al. (4) indicate that "hollowness" of good quality beans is due to overscalding, that is, overblanching, this laboratory has found hollowness occurring in good quality beans with extensive tissue rupture during freezing.

This investigation indicates that tissue rupture is not due to speed of freezing alone. A very important factor is the amount of free moisture in the package; for example, brine-packed vegetables and syrup-packed fruits frozen rapidly may exhibit more tissue rupture than dry packs frozen more slowly. Generally, however, the more slowly either a dry or a "wet" packed product is frozen, the greater is the tissue rupture. One other factor in the extent of tissue rupture is the stage of growth of the product. Succulent growing points with non-vacuolated cells are relatively more torn than those sections which are at a later stage of growth; for example, the tip of asparagus in comparison with the stalk.

In those fissures formed during freezing, the larger ice crystals are found in the more slowly frozen product. In very rapid freezing, as by immersion of the individual pieces in solutions at $-50°$ F. or even at $0°$ F., small ice crystals are in the cells and in such cavities as occur during freezing. In more slowly frozen products some ice may be found in the cell, but much more is found as a peripheral hull or layer of ice in the inter-cellular spaces and the fissures, proportionately larger, are also filled.

Some of the cavities may be so small as to be invisible without magnification, but with cooking the tears in the tissue do not close and are practically the same as in the frozen product.

SUMMARY

Tissue of asparagus requiring from under 2 minutes to 12 hours for freezing was examined after freezing and after storage periods. Very rapid freezing caused little tissue rupture. In brine and dry packs frozen at the same temperature, the brine packs required a shorter interval for freezing but the tissue had larger ice-filled lacunae. Increase of tissue rupture occurred with the addition of chilled solutions to the frozen product and generally the size of the lacunae increased with storage.

ACKNOWLEDGMENTS

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PRODUCTION METHODS AND THE KEEPING QUALITY OF CHURNING CREAM[1]

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Since the start of the war the flavour quality of Alberta creamery butter has retrogressed. This retrogression has been related to the lowered quality of the incoming raw cream which in turn is thought to be largely a reflection of the farm labour situation.

There is a wealth of knowledge of the relation of milk production methods to the keeping quality of fluid milk held at different temperatures. No such body of data relative to churning cream exists and there is practically no available information applicable to Alberta churning creams.

Churning cream production methods differ from market milk production techniques in that churning cream is usually delivered to the plant less frequently than is market milk and it conforms to a different set of grade standards. It has become customary to assume a necessity for either frequent delivery or mechanical refrigeration, if the highest grade of churning cream is to be attained. Either alternative is expensive.

For differentiation of grades the Alberta Cream Grade Standards depend on titratable acidity (expressed as percentage lactic acid), flavour and certain other characteristics.

Assuming clean flavour and uniform consistency, Special Grade, the highest grade applicable to churning cream, permits a maximum titratable acidity of 0.3 per cent, while for First Grade the titratable acidity may reach 0 6 per cent Creams of higher acidity or with certain flavour defects, etc., qualify for Second Grade and very objectionable flavours cause degrading to Off Grade.

It is generally assumed that Special Grade cream will churn into First Grade butter with a flavour score of not less than 40 and that First Grade cream will churn into First Grade butter with a flavour score of 39. The Cream Grade Standards are, thus, based on butter grading standards. In general, 40 score butter probably has superior keeping quality to 39 score butter. There is a mandatory minimum differential of 2 cents per pound in favour of Special Grade butterfat.

The purpose of the present study was to gather information, if possible, which will permit the producer to deliver churning cream of maximum quality with minimum expense. The study is limited to bacteriological considerations, does not include defects not induced by bacteria, and was conducted during the summer months only.

METHODS

Creams were collected in sterile containers at the point of origin, i.e., a local creamery (plant creams) and Farms A and B and transported immediately to the laboratory. They were then dispensed into sterile screw-cap medicine bottles and sterile covered test-tubes which were stored in water-baths held at the required temperatures. Accordingly, daily sampling was accomplished without disturbing any bulk cream.

Standard methylene blue reduction and plate counting techniques followed Standard Methods for the Examination of Dairy Products (A.P.H.A., 1943) except that plate incubation temperatures were 25° C. The titratable acidities (T.A.) were determined by the usual procedure and the grading followed official provincial practices. The stored creams were subjected to the various tests daily. The first few creams were stored at 40° F., 50° F. and 60° F. but the later samples were stored at 40° F., 45° F. and 50° F.

Farm A was producing a high quality cream from machine-drawn milk. Milk drawn by hand into sterilized utensils and separated by a sterilized separator was specially produced on two occasions for the purpose of this study.

Farm B was ordinarily producing, by hand milking, a cream of much lower quality and was experiencing difficulty in delivering Special Grade cream twice weekly. Cream 20 was morning cream produced as usual on this farm and held at 46° F. till it was collected in the evening. Cream 21 was from the evening milking on this farm, the only change being that all the utensils, including the separator parts, were sterile.

The points of origin and the initial reduction times, plate counts and titratable acidities of all the creams are given in Table 1.

Plant Creams   RESULTS

To study the keeping quality of creams of high bacterial content, a number of Special Grade creams were collected on delivery at a local creamery. In each case these were judged to be representative of the best creams received at the creamery on the day of collection. These were, of course, creams of unknown history and had been held on the producing farms for unknown but varying times at unknown but varying temperatures. The condition of these creams on delivery at the plant is given in Table 1 from which it is seen that acid production was well progressed except in creams 12 and 17. In these two creams it is probable that acid increase was just measurable. In no case did the cream remain in Special Grade at 45° F. for longer than 3 days (Figures 1 and 2).

There is a general trend toward lowered keeping quality as the bacterial content rises. There are some inconsistencies when different holding temperatures are compared, probably caused by differing bacterial floras and varying selective effects of the temperatures. Certainly, in practice, large bacterial populations do not permit infrequent delivery of the cream to the creamery and simultaneous attainment of Special Grade.
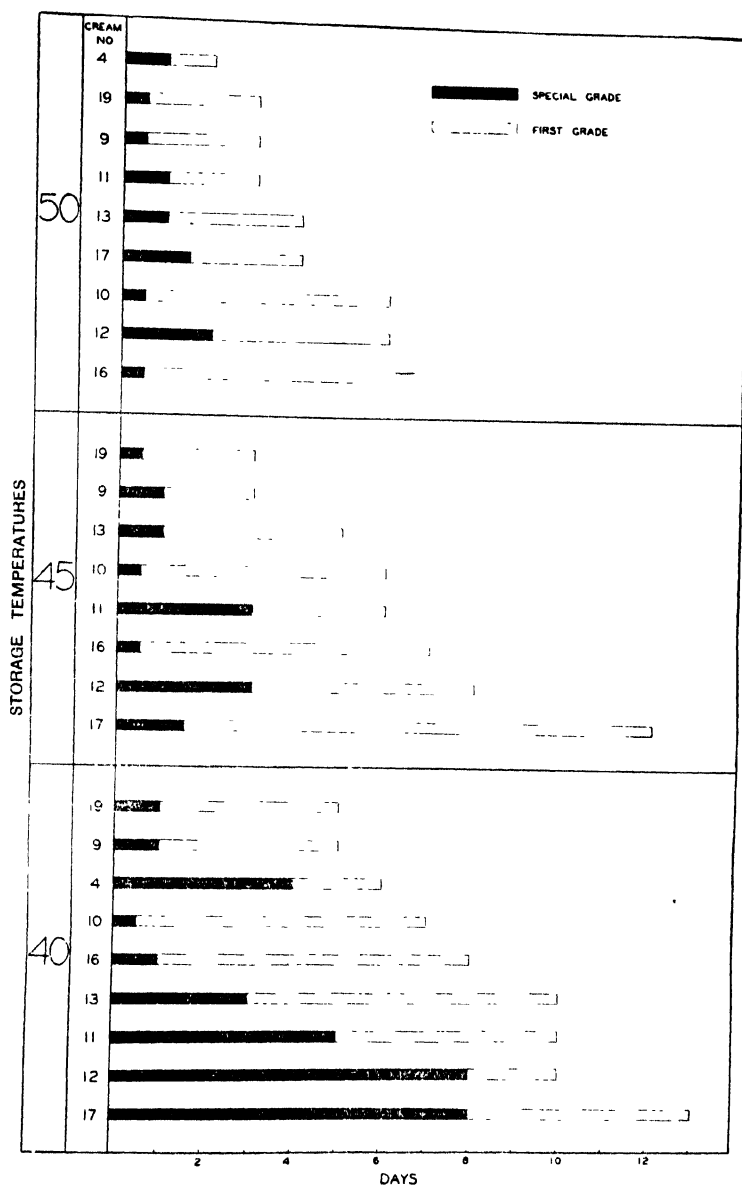
FIGURE 1   The number of days plant creams remained in Special and First Grades at various storage temperatures.
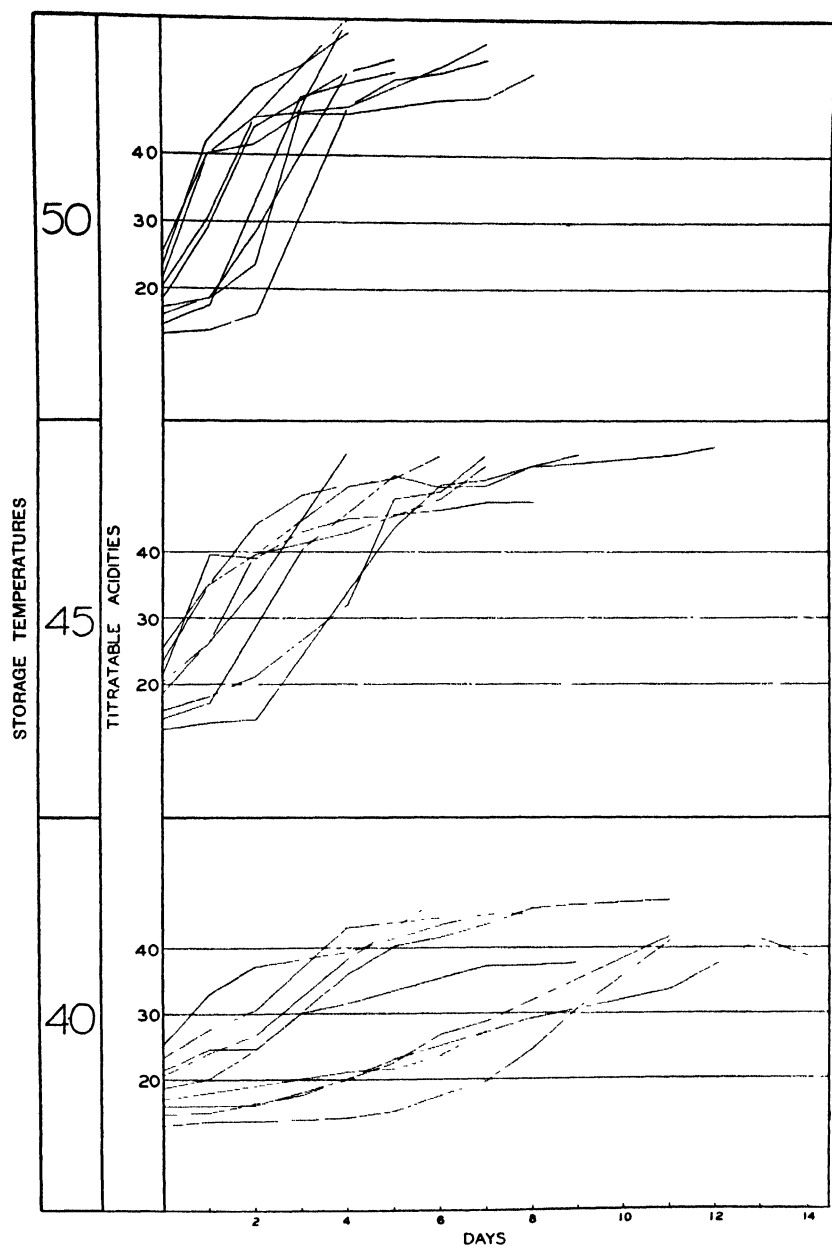
FIGURE 2.   The titratable acidities of plant creams stored at various temperatures.

TABLE 1.—ORIGIN AND INITIAL CONDITION OF THE CREAMS

Cream		T.A.	Reduction time in hours	Plate count
Source	Number			
Farm A	1	0.15	9	—
	2	0.125	>10	—
	3	0.130	7½	**3,000
	5	0.115	> 9	**320
	*6	0.125	>15	150
	*7	0.115	>10	1,500
	8	0.125	9	9,800
	14	0 110	7¾	10,700
	15	0.120	7¾	7,800
	18	0 095	8¾	3,000
Plant	4	0.170	½	—
	9	0.205	0	>30,000,000
	10	0 255	0	>30,000,000
	11	0.160	1	91,000,000
	12	0.130	4½	4,400,000
	13	0.185	0	177,000,000
	16	0.215	0	310,000,000
	17	0 145	0	77,000,000
	19	0.235	0	253,000,000
Farm B	20	0.115	1	5,500,000
	*21	0,115	10	12,500

* Sterile utensils.
** Plates incubated at 37° C.

Farm A Creams

This farm was known to be producing a cream of superior quality. The metal milk utensils were ordinarily sterilized just prior to use by immersion in water at or near the boiling point. The milking machine inflations were kept filled with a chemical disinfectant between milkings. The standards of general cleanliness were high. The substitution of laboratory autoclaved utensils did not raise the keeping quality of the cream from this farm (Creams 6 and 7).

The first few creams from this farm were stored at 40° F., 50° F. and 60° F. Even the creams of the lowest bacterial content had such poor keeping quality at 60° F. that twice weekly delivery of the cream would

not assure a grading of Special.   Lower temperatures are easily attained in this area without recourse to other than well-water refrigeration.   Therefore, 60° F. was adjudged not to be a practical storage temperature and experiments at this temperature were discontinued.

In earlier studies conducted by this Department it was found that the waters in the wells of this district are rarely, if ever, above 45° F., while not a few have temperatures of 40° F. or below.   Therefore, experiments on the keeping quality of creams stored at 40° F. were deemed to have practical value.

The results with Farm A creams are summarized in Table 2 and Figures 3 and 4.   These creams of low bacterial content graded as Special for at least six days when stored at 50° F. and for at least ten days when stored at 45° F.   When stored at 40° F. the creams remained in Special Grade for two to four weeks.   Such a long holding period is not to be recommended in practice, however, because it is conducive to the appearance of serious defects of non-bacterial origin, while the economics of the butter industry demand no such infrequency of delivery.

In some cases the day's grading placed a cream in First Grade while subsequently it graded Special.   There was noticeable inconsistency in this regard except that it never occurred during the first thirteen days of storage. The cause of this inconsistency was not determined but it may have been an artifact introduced by the non-representativeness of small lots of the original sparsely-populated creams.   If so, then this inconsistency would not appear in the grading of commercial bulk samples.

TABLE 2.—NUMBER OF DAYS FARM A CREAMS REMAINED IN SPECIAL AND FIRST GRADES AT VARIOUS STORAGE TEMPERATURES

Temperatures, °F.	Days			
	Special grade		First grade	
	Minimum	Maximum	Minimum	Maximum
60	2	4	5	8
50	6	9	9	15
45	10	17	12	19
40	17	30	17	>34

Farm B Creams

For assurance that utensils are responsible for the major bacterial contamination of ordinary churning creams, a farm producing a cream of inferior quality was chosen for study.   This farm was experiencing difficulty delivering Special Grade cream twice weekly, despite a holding temperature of 46° F.   Between a morning and an evening milking, all the utensils were sterilized and the results for both the morning and evening creams are shown in Table 3 and Figures 5 and 6.   Cream 20 (morning cream) was high in bacterial content and had very poor keeping quality.   Cream 21 (evening cream) was low in bacterial content and had excellent keeping quality.   The cream of superior quality was produced by no other change than to sterilize all the milk- and cream-contact surfaces.
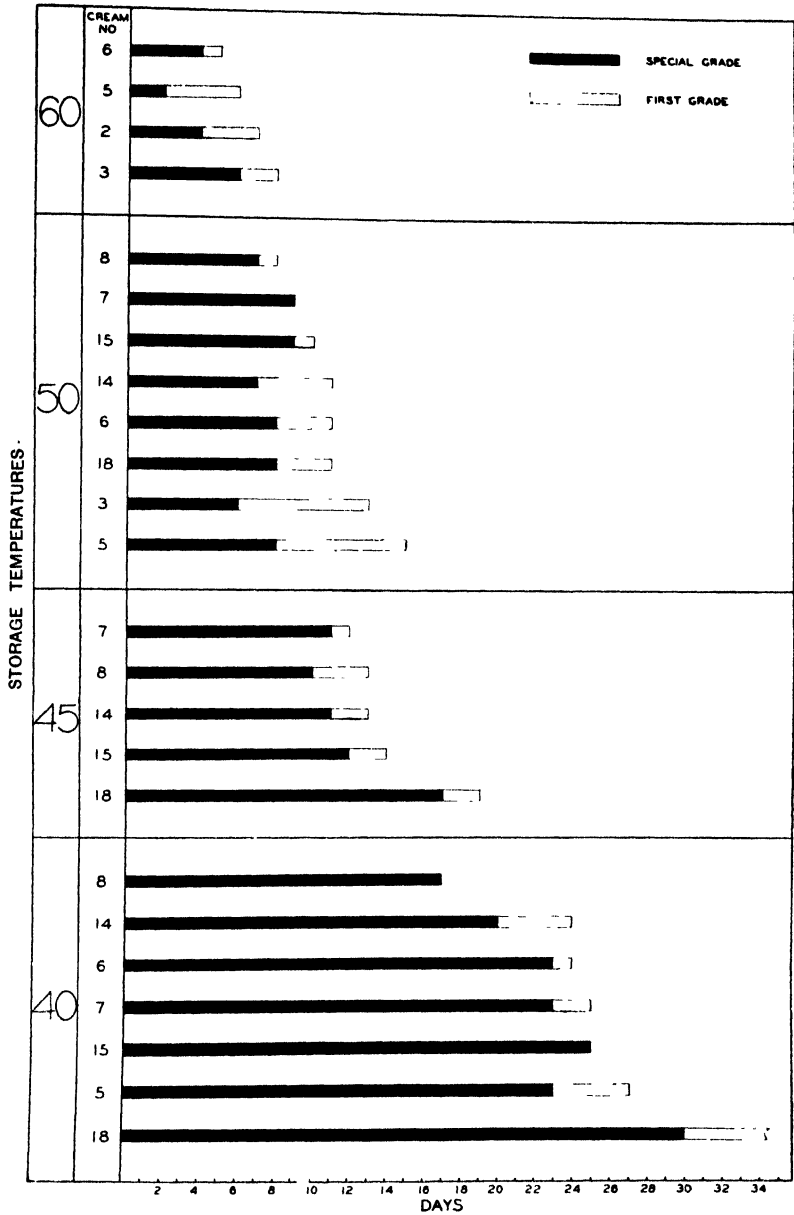
FIGURE 3.   The number of days Farm A creams remained in Special and First Grades at various storage temperatures.
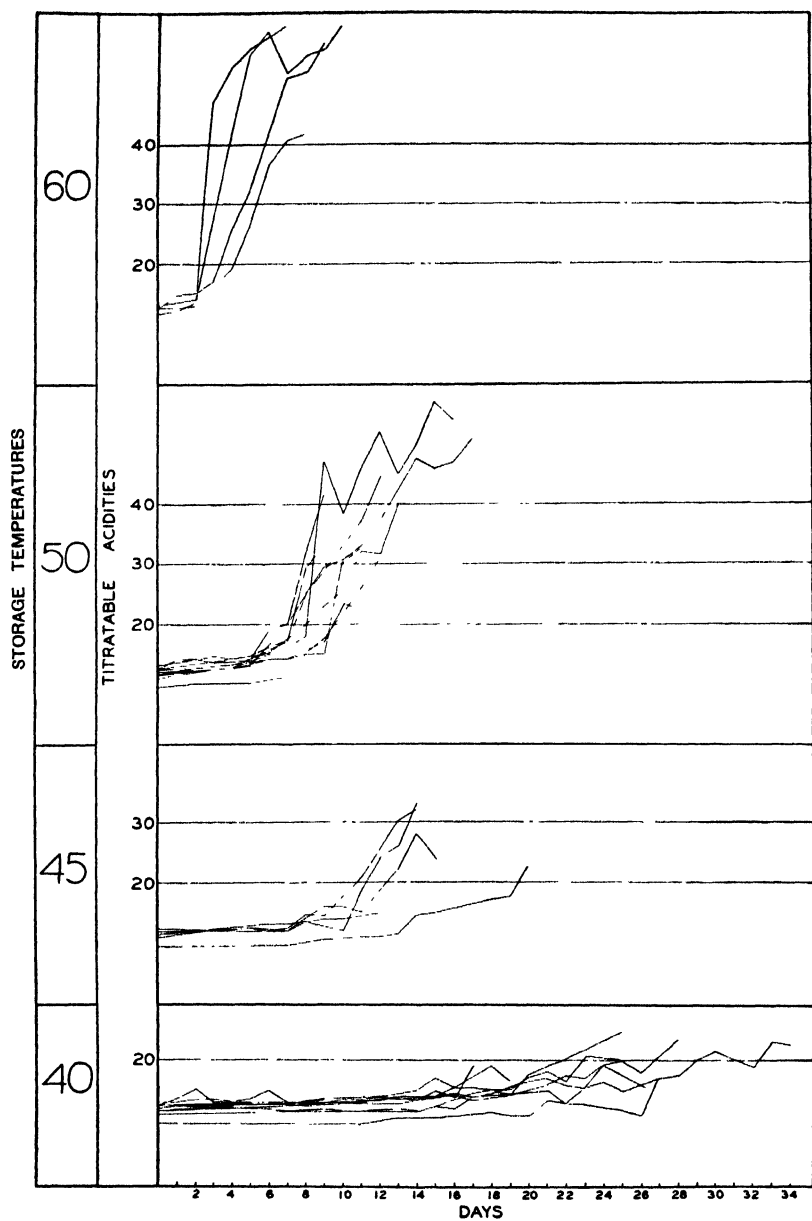
FIGURE 4. The titratable acidities of Farm A creams stored
at various temperatures.

TABLE 3.—NUMBER OF DAYS FARM B CREAMS REMAINED IN SPECIAL AND
FIRST GRADES AT VARIOUS STORAGE TEMPERATURES

Cream	Temperatures, °F.	Special	First
20	50	2	5
	45	3	7
	40	2	5
21	50	7	8
	45	11	15
	40	26	26

The Influence of Transportation on Keeping Quality

The above data show the keeping quality of creams held at constant temperatures representative of practical farm holding temperatures. During summer transportation from the farms to the creameries churning creams are almost always subjected to higher surrounding temperatures. Laboratory reproduction of practical conditions is difficult but it is easy to subject experimental creams to conditions more severe than are necessary in practice.

For the purposes of this study it was assumed that there is rarely, if ever, a necessity to transport Alberta churning creams for 10 hours in a surrounding atmosphere over 80° F. A 3-gallon can, a 5-gallon can and an 8-gallon can of churning cream of 33 per cent butterfat content at initial temperatures below 45° F. were stored for 10 hours in an atmosphere between 80° F. and 85° F. These creams rose in temperature an average of slightly more than 2° F. per hour as shown in Table 4 and Figure 7.
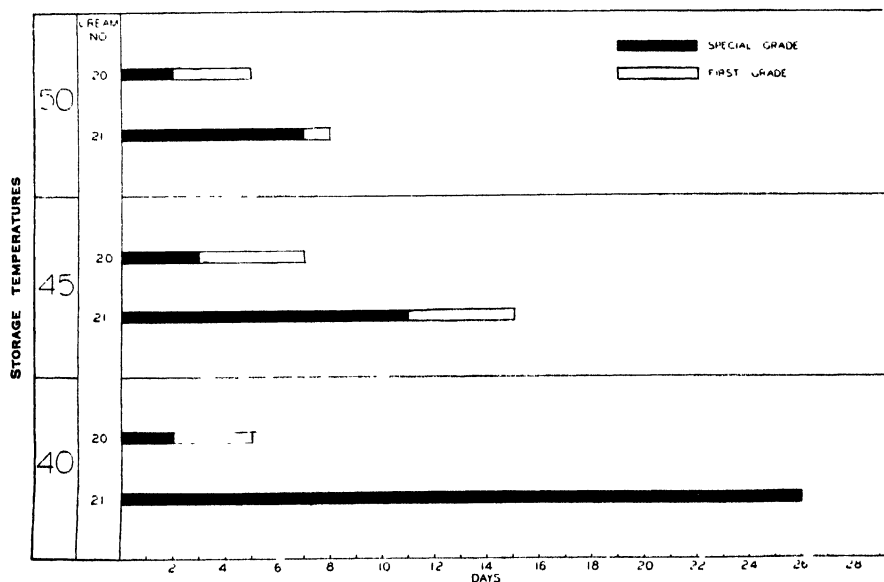

FIGURE 5.   The number of days Farm B creams remained in Special and First Grades at various storage temperatures.
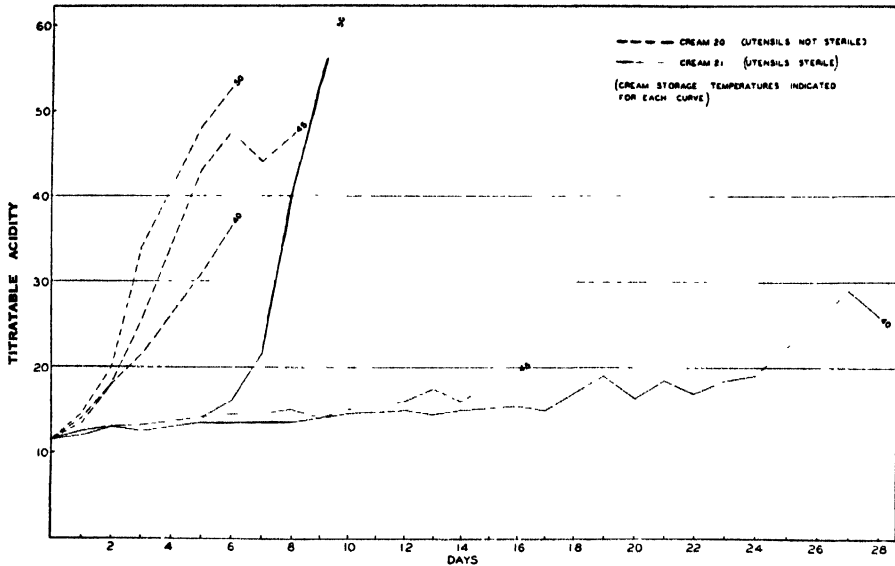
FIGURE 6.   The titratable acidities of Farm B creams stored
at various temperatures.

TABLE 4.—HOURLY TEMPERATURES OF CREAM HELD IN AN ATMOSPHERE ABOVE 80° F.

Time in hours	Temperatures in ° F.			
	3 Gallons	5 Gallons	8 Gallons	Atmosphere
0	42.5	41.0	42 0	82 5
1	48 0	46.0	45.5	85.0
2	52 0	50.0	49.5	83.5
3	54.5	52 0	52.0	81.5
4	57.0	55.0	54.5	82.5
5	59 0	57.0	56 0	82 5
6	60.5	59.0	57 5	81 5
7	62.0	60.0	59 0	82 0
8	63 5	61.5	60 5	82 0
9	64 5	62.5	62 0	82 5
10	66 0	64 0	63 0	83 5
Average increase /hour	2.35	2 3	2 1	

It is sometimes noticed that milk and cream held at low temperatures
for long periods deteriorate rapidly at higher subsequent temperatures.
To obtain information on this point, as related to transportation of high
quality churning creams, creams from Farm A were held at 50° F. for 5
days, at 45° F. for 7 days and at 40° F. for 10 days. They were then
incubated at 60° F. and 70° F. for ten hours and the grades and titratable
acidities determined hourly as reported in Table 5.

It is seen that creams of this quality withstand rigorous treatment
without serious deterioration.   Therefore, creams of low bacterial content
have an inherent "factor of safety" sufficient to maintain Special Grade on
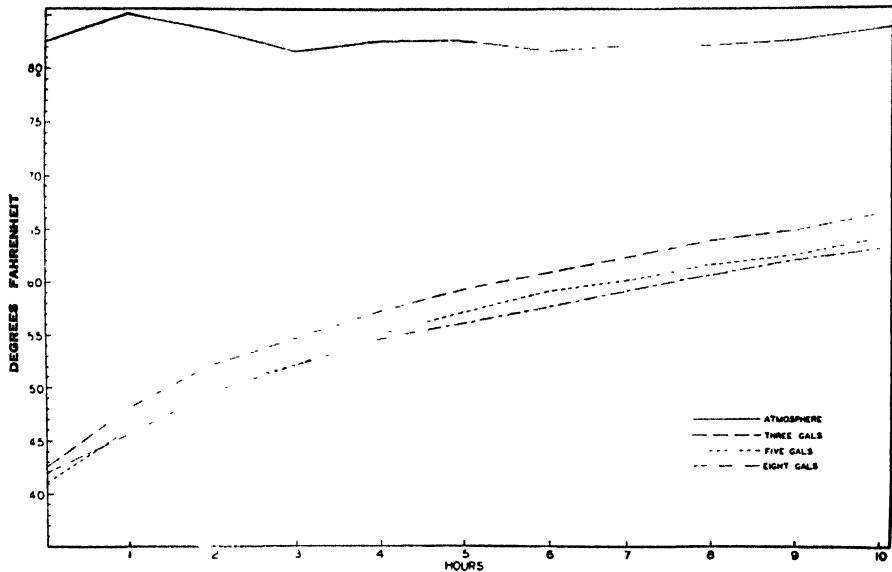
FIGURE 7.   Temperatures of cream held in an atmosphere above 80° F.

delivery at the creamery twice weekly if stored at 50° F. and once weekly if stored at 45° F. or below, assuming reasonable care during transportation from the farm to the creamery.

Titratable Acidity

Titratable acidity as used in the dairy industry assumes that the only acid present in milk or cream is lactic acid.   This assumption is probably never correct and the test is misleading in proportion to the kinds and amounts of other acids present.   Lactic acid is present as the principal acid and in large amounts only as a result of the action of the lactic acid bacteria.   In this area milk or cream of very low bacterial content contains few cells of lactic acid bacteria.   This group appears to be introduced into the milk or cream in large numbers solely by non-sterile utensils.   Temperatures below 50° F. are not favourable to the growth of these bacteria.   On the other hand these low temperatures permit the growth of other species of bacteria some of which are acid forming but which produce little or no lactic acid, while some are non-acid forming.

The only criterion applicable in these studies for the differention of Table and Special Grade creams was titratable acidity.   The titratable acidity of Table Grade cream must not be over 0 2 per cent.   That titratable acidity had very limited value as a grading criterion of the creams of low bacterial content is illustrated by the fact that their grade deterioration from Special to First took place while the titratable acidities were below 0.20 (Figures 2, 4, 6).   The degrading was on a basis of either non-acid flavour defects or in some cases non-lactic acid flavour defects. In many of the creams degrading was directly from Special to Second Grade, or First Grade was maintained for very short periods.   It is apparent

TABLE 5.—EFFECT OF HIGHER SUBSEQUENT TEMPERATURES ON STORED CREAMS

Stored at	40° F. for 10 days				45° F. for 7 days				50° F. for 5 days			
Incubated at	60° F.		70° F.		60° F.		70° F.		60° F.		70° F.	
Hours	T.A.	Grade	T.A.	Grade	T.A.	Grade	T.A.	Grade	T.A.	Grade	T.A.	Grade
0	0.120	Special	0.120	Special	0.130	Special	0.130	Special	0.110	Special	0.110	Special
1	0.120	Special	0.125	Special	0.125	Special	0.130	Special	0.120	Special	0.130	Special
2	0.120	Special	0.125	Special	0.130	Special	0.125	Special	0.120	Special	0.125	Special
3	0.120	Special	0.130	Special	0.125	Special	0.130	Special	0.130	Special	0.130	Special
4	0.125	Special	0.130	Special	0.120	Special	0.140	Special	0.125	Special	0.125	Special
5	0.120	Special	0.135	Special	0.125	Special	0.125	Special	0.120	Special	0.140	Special
6	0.130	Special	0.140	Special	0.120	Special	0.160	Special	0.120	Special	0.145	Special
7	0.125	Special	0.140	Special	0.130	Special	0.145	Special	0.115	Special	0.150	Special
8	0.125	Special	0.145	Special	0.135	Special	0.135	Special	0.130	Special	0.145	Special
9	0.130	Special	0.160	Special	0.140	Special	0.145	Special	0.130	Special	0.145	Special
10	0.135	Special	0.170	Special	0.140	Special	0.150	Special	0.140	Special	0.150	Special

TABLE 6.—EFFECT OF PLATE INCUBATION TEMPERATURE ON THE PLATE COUNTS OF STORED CREAMS

Cream	Stored		Plate incubation temperatures					
	Days	Temperature	37° C., 2 days	25° C., 5 days	15 6° C., 7 days	10° C., 8 days	7.2° C., 11 days	4.5° C., 14 days
3	7	40° F.	2,750	70,000	>30,000			
3	7	50° F.	165,000,000	135,000,000	130,000,000			
5	8	40° F.	7,000	100 000	100,000			
5	8	50° F.	520,000,000	548,000,000	595,000,000			
18	15	40° F.	1,325,000	4,250,000		6,000,000	7,000,000	3,000,000
18	15	50° F.	49,000,000	73,500,000		78,000,000	68,000,000	8,500,000

that, as cream supplies improve and holding temperatures are lowered and holding periods lengthened, the titratable acidity test is destined to play a more limited role in cream control.

The fact that Special Grade cream commands the highest price paid for churning cream has led to considerable confusion in the common conception of the bacteriological condition of such cream. The standard for this Grade permits a titratable acidity up to 0.30. A cream of this acidity is sour to the taste and has a total bacterial content approaching the maximum number possible to grow in cream (Table 1). Judged by the criteria usual in the market milk industry, such a cream has long since passed into the rejectable class—it is "good" cream only in that it will churn into First Grade butter.

BACTERIOLOGICAL TESTS

Of the bacteriological tests used for the routine grading of raw fluid milks, the reduction tests are the simplest and most nearly fool-proof. No other test is sufficiently cheap for routine application to raw churning creams. The theoretical methylene blue reduction time of a milk is zero, or nearly so, when the milk contains about 100 million bacteria per ml. At the time a milk shows any increase in acidity measurable by the titratable acidity test, the bacterial content of the milk will approach 100 million cells per ml. It is seen that the time differentials are outside the scope of the reduction test by the time there is a measurable increase in acidity. The same relation probably holds approximately true for the various resazurin tests. Therefore, the reduction tests do not appear to have a routine place in the quality control of churning creams at the point of delivery.

The plate count is widely applied in the milk industry and, at first sight, might seem to be applicable to the problem under discussion here. But, apart from cost considerations, one factor alone complicates plate count results to the extent that much research would probably have to be done before adoption of the test is possible for this purpose. This complication arises because of the varying but long holding periods at low but varying temperatures in the churning cream industry. Assuming that there is one ideal medium for supporting bacterial growth—which there is not—there is still no single ideal temperature for incubation of the plates. This is illustrated by the data in Table 6.

But there appears to be no present purpose in the routine application of bacteriological tests to raw churning creams at the point of delivery. The highest official grade recognized for such cream is Special and specifications for this grade permit creams that are quite sour to the taste. By taste alone much finer differentiations are possible than are required by this grade standard. Differentiation by bacteriological tests will not be practical until very considerably higher flavour standards for butter are demanded by the public.

On the other hand this was an investigation of the growth of bacteria in creams held at low temperatures with acid production and flavour changes as the measures of such growth. It was deemed desirable to supplement these tests with the standard bacteriological techniques. Therefore, methylene blue reduction times and plate counts were determined daily for most of the creams throughout their storage periods.

The methylene blue reduction test was found to have very limited value because the reduction times were zero usually before there was any increase in titratable acidity and always long before the creams were degraded. This was despite the encouragement of a psychrophilic flora by low storage temperatures, while the test incubation temperature was 37° C. It may be that during the long storage there was considerable fixation of the oxygen dissolved in the cream. Nevertheless, no seriously anomalous results were observed and the reduction times of Farm A creams paralleled storage times and temperatures.

Plate counts of Farm A creams were found to be applicable throughout the entire grading period but there were many irregularities. In general the counts paralleled the storage times and temperatures. The daily reduction time and plate count curves for cream 18 are presented in Table 7. From Table 8 it will be noted that there is a tendency for the creams of low bacterial content stored at 40° F. to be degraded while the plate counts were still comparatively low. This is because degrading was not the result of the action of lactic acid bacteria. However, there can be no doubt that extensive bacterial growth took place in these creams at 40° F.

TABLE 7.—GROWTH OF BACTERIA IN CREAM 18 STORED AT VARIOUS TEMPERATURES

| Days of storage | Storage temperatures | | | | | |
| | Plate counts | | | Reduction times (hours) | | |
	40° F.	45° F.	50° F.	40° F.	45° F.	50° F.
0	3,000	3,000	3,000	8¾	8¾	8¾
2	—	<4,000	<25,000	—	>4	>4
5	—	400,000	11,000,000	—	6	¼
7	11,800	8,600,000	69,000,000	>9	2¼	0
8	—	18,750,000	127,000,000	—	1¼	
9	189,000	74,000,000	137,000,000	>4	¾	
11	362,000	62,000,000	459,000,000	9½	¼	
12	—	58,000,000	510,000,000	—	0	
13	—	74,000,000		6		
14	1,550,000	240,000,000		4¼		
15	4,250,000	73,500,000		4		
18	46,000,000	220,000,000		1¼		
19	19,000,000	114,000,000		2		
20	72,000,000	23,650,000		0		
22	65,000,000					
25	104,000,000					
27	124,000,000					
29	187,000,000					
33	263,000,000					

TABLE 8.—PLATE COUNTS OF CREAMS ON THE LAST DAY ON WHICH THEY GRADED SPECIAL

Origin	Cream	Plate counts		
		Storage temperature		
		40° F.	45° F.	50° F.
Farm A	5	154,000,000	—	70,000,000
	6	66,000,000	—	32,000,000
	7	26,000,000	79,000,000	206,000,000
	8	<20,000,000	70,000,000	350,000,000
	14	36,000,000	310,000,000	315,000,000
	15	66,000,000	300,000,000	510,000,000
	18	187,000,000	73,500,000	127,000,000
Plant	11	195,000,000	200,000,000	<600,000,000
	12	350,000,000	91,000,000	127,000,000
	13	200,000,000	—	—
	16	310,000,000	—	—
	17	320,000,000	219,000,000	410,000,000
	19	345,000,000	—	—

Types of Defects

Some creams had shortened keeping times because of the appearance of defects believed not to be caused by bacterial action. Oxidized flavours catalysed by dissolved metals from utensils in recognizably poor condition and rancid flavours may be mentioned. Such defects are not considered in this communication other than to state that, because of them, grades were sometimes lowered much sooner than would otherwise have been the case.

As was expected, there were encountered some bacterially-induced defects which are not common in commercial churning creams. As commercial creams are improved these defects are likely to become more prominent. Two such defects were sufficiently impressive to merit mention.

Many of the creams of low bacterial content stored for long periods at the lowest temperatures developed a strong bitterness suggestive, not of the peptones, fatty acids or aldehydes, but of the alkaloids. Isolations were made of short, gram negative rods capable of producing this defect markedly in skim milk at temperatures varying from 40° F. to 98.6° F.

A few creams lost grade because of a sharp acid flavour, although the titratable acidity was relatively low. This defect appeared to be more prevalent at the higher than at the lower storage temperatures.

SUMMARY AND CONCLUSIONS

1. Summer churning cream of low bacterial content at the time of production on the farm is achieved by the same procedure necessary for the production of market milk of low bacterial content. The most important step in this procedure is to permit the milk and cream to come into contact with only sterile surfaces. Therefore, *all* utensils must be sterilized.

2. Special Grade may be attained with twice weekly delivery when the storage temperature of cream of low bacterial content is 50° F., and with weekly delivery when the storage temperature is not over 45° F. Five degrees difference in storage temperature makes a marked difference in the keeping quality of the stored creams within the range of 40° F. to 50° F. These temperatures are attainable in the Edmonton area by the proper use of well-water as the coolant.

3. The long storage of such creams at temperatures of 50° F. or below will probably result in bacterially-induced flavour defects not now very common in the churning cream industry.

4. The titratable acidity test in these circumstances may become of very limited value as a differential criterion in grading. Frequently it is misleading.

5. The reduction test and the plate count do not appear to offer any advantages in the routine control of churning creams when applied at the point of delivery.

CANADIAN ERUCIC ACID OILS

I. REFINING AND BLEACHING[1]

By N. H. GRACE[2]

Abstract

Dark colored commercial erucic acid oils from rapeseed, and from mustard seed screenings (*Brassica* species), have been subjected to various refining and bleaching treatments. Superfiltrol bleaching clay was superior to Neutrol clay or an unactivated Manitoba bentonite. Lecithin-free, partially alkali refined, and alkali refined oils were bleached readily by heating for 20 min. at 100° C., as little as 2% clay yielding oils similar in color to commercial table oils. Crude oils could be bleached without preliminary refining treatment if the temperature were raised to 200° C., though some increase in free fatty acid occurred. With Stanolax as a color standard, relative transmissions at a wave length of 440 mμ were 4.5% for crude rapeseed oil, 5 0% for alkali refined rapeseed oil, and 58, 78, and 91%, respectively, for the alkali refined oil bleached with 1, 2, and 4% Superfiltrol. The behavior of mustard seed oils was closely similar though more bleaching clay was required. For both rape and mustard, spectrophotometric analyses indicated generally similar transmissions for crude, lecithin-free, and partially alkali refined oils in the range from 225 to 500 mμ, while fully alkali refined oils showed reduced transmissions in the triene region (260 to 280 mμ) with slightly increased transmission over the range from 320 to 500 mμ. Bleached oils were characterized by high transmissions in the range above 320 mμ.

Introduction

The erucic acid oils produced in Canada are obtained from rapeseed and from mustard seed varieties (*Brassica* species) screened from the western Canadian wheat crop. The commercially expelled oils are usually very dark in color and generally unattractive in appearance. The undesirable color of rapeseed oil has been attributed to the presence of chlorophyll in the common black Argentine rape grown in Canada where it ripens unevenly. Whatever the cause, improvement in color is a necessary antecedent to any edible use and to most industrial uses.

Although erucic acid oils have been widely used for edible purposes in India and China, their use as food has been relatively inconsequential in North America. Verbal reports had indicated that effective color removal was difficult, alkali refined oils tending to yield green products on hydrogenation. These statements were confirmed by hydrogenation of commercially available refined and bleached rapeseed oil, which developed the reported dis-

coloration. A study of the recent literature on German wartime technology shows that rapeseed oil was their chief source of edible fat (3). The present world shortage of fats and the possibility of large scale Canadian production of erucic acid oils were among the considerations leading to this study of processing conditions.

Materials and Methods

Crude rape and mustard oils were obtained from a commercial source in Moose Jaw, Saskatchewan; their general characteristics are given in Table I. These were refined and bleached in various ways, and the resulting oils were examined with special reference to color and free fatty acid.

TABLE I

CHARACTERISTICS OF CRUDE ERUCIC ACID OILS

	Rapeseed oil	Oil from mustard seed screenings
Refractive index, 25° C.	1.4715	1.4735
Saponification value	179	188
Free fatty acid content (as oleic), %	0.4	0.5
Iodine value	103	118
Color F.A.C. Standard Colors, not darker than number	21	27
Relative transmission with Stanolax as standard in Evelyn photoelectric colorimeter, % transmission at 440 mμ 660 mμ	4.5 32.5	4.8 15.0

Conventional alkali refining was conducted in accordance with A.O.C.S. procedures set down for the refining of soybean oils (1). Partial alkali refining involved a similar procedure with the use of an amount of 6% sodium hydroxide solution stoichiometrically equivalent to the free fatty acid content; these oils are hereafter referred to as 'alkali treated'.

A number of rapeseed oil samples were processed as follows: alkali refined by conventional methods; subjected to five water washings at 100° C. to remove lecithin in accordance with reported German procedure (3); alkali treated to yield an oil of about the same free fatty acid content as the lecithin-free sample; and subjected to steam distillation for one hour at 200° C. The mustard seed oil was subjected to alkali treatment and to conventional alkali refining.

The bleaching clays used were two activated, commercial preparations, Superfiltrol and Neutrol (from the Filtrol Corporation, Los Angeles, Calif.), and a Pembina Manitoba bentonite, which was unactivated. The moisture contents of these clays varied from 3.1 to 4.2%. A few trials made use of the American Oil Chemists Society's official Fuller's earth.

The bleaching operation was conducted in equipment comprised of three heating mantles furnished with 500 ml. three-necked flasks. Each experiment was made on 100 gm. of oil. Bleaching clay was added to the cold oil and the free space of the flask flushed with carbon dioxide throughout the heating period. Heating of the mantles was controlled by means of variable transformers set to bring the temperature to the desired point in approximately 10 min., and manually controlled thereafter. Stated temperatures were maintained within $\pm 3°$ C. When the desired heating period had elapsed, the mantle was lowered, stirring stopped, and the oil filtered rapidly by suction through a Büchner funnel. Oil samples were stored under an atmosphere of carbon dioxide in ordinary glass bottles in the diffuse light of the laboratory.

Spectrophotometric absorption curves were made on both crude and refined oils with the Model D-11 Beckman instrument, 1 gm. of oil being made up to a volume of 500 ml. with normal heptane and an aliquot taken for measurement in a 1 cm. quartz cuvette. The color of crude, refined, and variously bleached oils was compared by means of the Evelyn photoelectric colorimeter, with Stanolax, a white mineral oil, as the reference medium. Relative transmissions were determined at wave lengths of 440 and 660 mμ, respectively. These wave lengths were selected because it has been shown that chlorophylls A and B show absorption maxima in these regions (2). The free fatty acid content of oil samples was determined and expressed as per cent oleic acid.

Results

Spectrophotometric analyses of rape and mustard oils confirmed their general similarity. The curves A to D of Fig. 1 describe the changes in extinction coefficients over the range from 225 to 500 mμ, for crude, alkali treated, alkali refined, and alkali refined and bleached mustard oils. The alkali refined oil (C), compared with the crude oil (A), showed higher extinction values in the triene region (260 to 280 mμ), but above 310 mμ the values fell below those for the crude oil. Extinction coefficients for alkali treated oil (B) coincided with those for the crude oil (A) until the wave length reached 300 mμ, and thereafter the points fell approximately between those of Curves A and C. Alkali refined oil bleached with 4% Superfiltrol clay (D), in comparison with the unbleached oil (C), showed slightly decreased extinction coefficients at 230 to 240 mμ but increased extinction coefficients at 260 to 280 mμ; otherwise the values were virtually identical to a wave length of 320 mμ. From this point the coefficients fell to an extremely low value, indicating inappreciable absorption beyond 350 mμ.

Closely similar results were obtained from the spectrophotometric examination of rapeseed oil. The crude, the lecithin-free, and the alkali treated oils gave extinction coefficients that differed slightly over the entire range. The alkali refined oil showed enhanced extinction values in the triene region but lower values above 330 mμ. Bleached rape oil followed the pattern already described for bleached mustard oil.

Bleaching Clays

It is apparent from the data of Tables II, III, and IV, that Superfiltrol is a better bleaching agent than Neutrol, and very much better than unactivated Pembina Manitoba bentonite. The three clays showed different bleaching
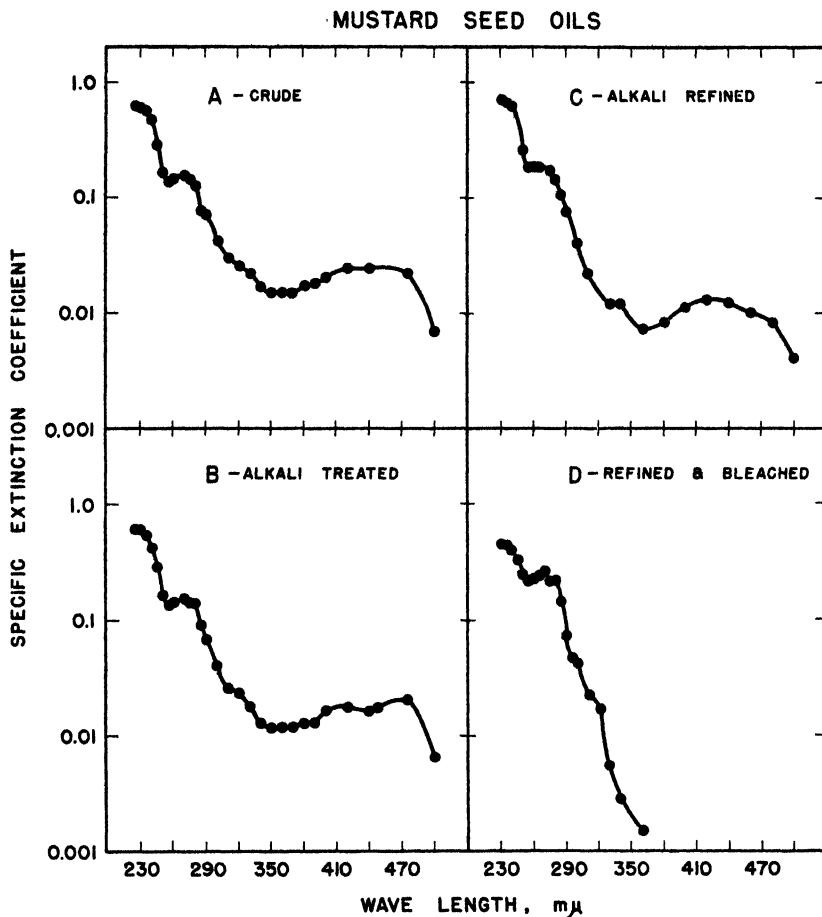

FIG. 1. *Ultraviolet absorption curves for normal heptane solutions of variously refined mustard seed oils.*

properties when judged by transmission at 660 mμ and the differences were accentuated with transmission at 440 mμ. Also, Superfiltrol did not appear to increase the free acid content when used at 4% for a heating period of 20 min. at 100° C. Superfiltrol was therefore used throughout the remainder of the study.

Effects of Bleaching Conditions

The results of Table V (*A*) indicate that increase in the duration of heating from 20 to 80 min. at 100° C. with 4% Superfiltrol had little effect on the relative transmission of the oil. There was some indication that the free

TABLE II

EFFECT OF DIFFERENT CLAYS ON THE BLEACHING OF RAPESEED OIL

(Oil heated for 20 min. at 100° C. with 4% of clay)

Clay	Crude oil			Alkali treated oil			Lecithin-free oil		
	F.F.A.,	Rel. transmission		F.F.A.,	Rel. transmission		F.F.A.,	Rel. transmission	
	%	660 mμ	440 mμ	%	660 mμ	440 mμ	%	660 mμ	440 mμ
None	0.42	32.5	4.5	0.24	39.5	4.6	0.29	32.5	4.9
Pembina bentonite	0.58	59.0	4.5	0.33	88.2	18.1	0 23	90.5	13.5
Neutrol	0.40	85.8	9.8	0.32	95.0	47.3	0.20	95.2	34.1
Superfiltrol	0.38	89.0	16.3	0.24	94.9	54.4	0.23	96.1	60.0

TABLE III

EFFECT OF DIFFERENT CLAYS ON THE BLEACHING OF OIL FROM MUSTARD SCREENINGS

(Oil heated for 20 min. at 100° C. with 4% clay)

Clay	Crude oil			Alkali treated oil		
	F.F.A., %	Rel. transmission		F.F.A., %	Rel. transmission	
		660 mμ	440 mμ		660 mμ	440 mμ
None*	0 49	15.0	4 8	0 25	18.1	4.0
Pembina bentonite	0 52	27 9	5 0	0 31	73 8	5.3
Neutrol	0 50	61 0	5 0	0 19	82 9	15 0
Superfiltrol	0 50	80 5	6 0	0 36	91 7	20 5

* *The corresponding oil without any bleach.*

fatty acid content of crude rapeseed oil rose slightly with the longer heating periods, but this was not noted for alkali treated oils.

The results of Tables IV, V (*B*), and VI indicate very marked temperature effects, especially evident for transmissions at 440 mμ, which appeared to pass through a maximum at about 200° C. This effect was noted in the crude rape, steam treated rape, and mustard oils, but not in the alkali treated materials. The crude rape oil subjected to steam treatment (Table IV) showed little change in transmission below 175° C. This sample of oil (bleached at 175° C.) was the only one in the entire investigation that was difficult to filter.

Free fatty acid content tended to increase appreciably with an increase in bleaching temperatures, the effect being substantially greater with crude than with alkali treated oils.

Effects of Superfiltrol Concentration

The effects of Superfiltrol over a wide range (2 to 12%) on bleaching for a 20 min. period are illustrated by the data in Tables VII to IX. Crude rape

TABLE IV

EFFECTS OF CLAYS AND TEMPERATURES ON THE BLEACHING OF CRUDE RAPESEED
OIL SUBJECTED TO STEAM DISTILLATION FOR ONE HOUR

(Clay at 4% concentration with a 20 min. heating period)

Effect of different clays, at 100° C.

Clay	F.F.A., %	Rel. transmission	
		660 mμ	440 mμ
None*	0.46	14.9	4 0
Pembina bentonite	0.35	27.0	3.8
Neutrol	0.30	40.0	4.0
Superfiltrol	0.29	44.7	4.2

Effect of temperature of bleaching with Superfiltrol

Temp., ° C.	F.F.A., %	Rel. transmission	
		660 mμ	440 mμ
100	0.29	44.7	4.2
125	0.33	55.2	5.0
150	0.28	57.1	6 0
160	0.38	84 0	22 8
175**	0.53	98.5	54 0
175**	0.69	98 5	52.0
200	0.74	100 0	59 5
225	1.11	98.7	49.3

*   * Steam treated rape oil, no bleach.
  ** Duplicates done at different times.

oil, bleached at a temperature of 200° C. (Table VII), required at least 4%
clay to effect substantial improvement in transmission at 440 mμ.   As much
as 12% Superfiltrol with crude oil yielded products inferior in color to those
obtained with 6% clay on the alkali treated and lecithin-free oils.   The crude
rape oil showed very marked increase in free fatty acid content with increasing
concentrations of bleaching clay.   Since all samples of alkali treated, lecithin-
free, and fully alkali refined oils showed comparatively little change in free
fatty acid, the effect on crude oil may be largely attributed to the high bleach-
ing temperature.

The data for alkali treated mustard oil (Table VIII) indicate poorer bleach-
ing than for comparable rape oil (Table VII), the difference being particularly
evident for transmissions at 440 mμ.   Alkali refined oils (Table IX) showed
substantially better bleaching than alkali treated oils (Tables VII and VIII),
the extent of the improvement being greater for rape than for mustard.   It is
interesting to note that 10% Superfiltrol was required with alkali treated
mustard oil to equal the bleaching achieved by 4% clay on alkali refined oil.

TABLE V

THE EFFECT OF TIME OF HEATING AND THE EFFECT OF TEMPERATURE
ON THE BLEACHING OF RAPESEED OIL

Condition	Crude oil			Alkali treated oil		
	F.F.A., %	Rel. transmission		F.F.A., %	Rel. transmission	
		660 mμ	440 mμ		660 mμ	440 mμ

A. Effect of time of heating. (Oil heated at 100° C. with 4% Superfiltrol)

Time of heating, min.						
20	0.38	89.0	16.3	0.24	94.9	54.4
40	0.56	92.0	8.2	0.18	97.0	66.0
60	0.56	93.0	15.5	0.20	95.0	59.3
80	0 62	93.0	24 8	0.18	100.0	61.0

B. Effect of temperature. (Oil heated for 20 min. with 4% Superfiltrol)

Temperature, ° C.						
100	0.38	89.0	16.3	0.24	94.9	54.4
125	0.35	94.8	25.8	0.23	98.5	68.0
150	0.47	94 0	33.5	0 28	98.0	62.0
175	0.50	93.2	33 8	0.90	99.0	66.3
200	0 57	99 0	53.5	0.70	99.1	65.0
225	1 07	98 0	47.7	—	—	—
250	1 61	97.2	33.0	—	—	—

TABLE VI

THE EFFECT OF TEMPERATURE ON THE BLEACHING OF MUSTARD SEED OIL

(Oil heated for 20 min., with 4% Superfiltrol clay)

Temperature, ° C.	Crude oil			Alkali treated oil		
	F.F.A., %	Rel. transmission		F.F.A., %	Rel. transmission	
		660 mμ	440 mμ		660 mμ	440 mμ
100	0 50	80 5	6 0	0.36	91.7	20.5
125	0.45	86 1	6.0	0.52	94.9	21.9
150	0.50	86 2	8.6	0 64	94.5	31.9
175	0.58	88 5	10 9	0 79	95 0	36.9
200	0.84	67.8	5 5	0.80	95.9	37.1

It is also evident from Table IX that 4% Superfiltrol was required to bleach mustard oil to the extent achieved by 1% with rape oil.

Results of bleaching with 4% Standard Fuller's earth of the American Oil Chemists' Society are included in Table IX for both the rape and mustard seed oils for purposes of comparison.

TABLE VII

EFFECT OF SUPERFILTROL CONCENTRATION ON THE BLEACHING OF RAPESEED OIL

(Oil heated for 20 min.)

Clay, %	Crude oil (200° C.)			Alkali treated oil (100° C.)			Lecithin-free oil (100° C.)		
	F.F.A., %	Rel. transmission		F.F.A., %	Rel. transmission		F.F.A., %	Rel. transmission	
		660 mμ	440 mμ		660 mμ	440 mμ		660 mμ	440 mμ
1	0.24	35.8	4.8	0.18	89.8	9.4	0.48	88.7	8.3
2	0.29	72.5	7.2	0.20	92.5	31.0	0.42	94.8	29.1
4	0.57	99.0	53.5	0.24	94.9	54.4	0.23	96.1	60.0
6	0.96	100.2	60.1	0.31	98.0	71.0	0.40	98.9	71.9
8	1.11	100.2	68.6	0.25	98.5	78.5	0.43	100.0	79.3
10	1.29	100.2	64.4	0.22	99.2	84.0	0.44	100.0	85.5
12	1.54	99.5	67.0	0.52	98.8	85.6	0.41	100.0	84.0

TABLE VIII

EFFECT OF SUPERFILTROL CONCENTRATION ON THE BLEACHING OF
ALKALI TREATED MUSTARD OIL

(Heated for 20 min. at 100° C.)

Clay concentration, %	F.F.A., %	Relative transmission	
		660 mμ	440 mμ
0*	0.25	18.1	4.0
1	0.33	53.5	5.1
2	0.40	76.1	7.0
4	0.36	91.7	20.5
6	0.32	94.1	34 4
8	0.38	100.0	49.5
10	0.45	97.9	55.9
12	0.55	100.0	60 3

* Alkali treated oil without any bleach.

Effects of Active Carbon, Added Water, and Modifications in Procedure

It is apparent from Table X that active carbon was somewhat more effective when used along with Superfiltrol (Treatments 1 and 2), but reference to Table VII shows that 4% Superfiltrol alone yielded oil with approximately the same transmission.

Treatment 3 used a five minute heating period at 100° C. prior to addition of bleaching clay. The resulting oil had poorer transmission at 440 mμ than oil from ordinary bleaching (Table V). This suggests that readily volatilizable material did not affect the activity of the clay. The addition of 1 and 2% water (Treatments 4 and 5) showed no appreciable effect. Treatments 6 and 7 involved two 2% Superfiltrol bleachings of mustard oils. But results on comparable oils subjected to one 4% bleach (Tables VII and VIII)

indicate that the double procedure was slightly less effective than the simpler, single operation.

TABLE IX

EFFECT OF CONCENTRATION OF SUPERFILTROL ON THE BLEACHING OF
FULLY ALKALI REFINED RAPE AND MUSTARD OILS

(Oil at 100° C. for 20 min.)

Superfiltrol concentration, %	Rapeseed oil			Mustard seed oil		
	F.F.A., %	Rel. transmission		F.F.A., %	Rel. transmission	
		660 mμ	440 mμ		660 mμ	440 mμ
0	0.068	72 5	5 0	0.069	52.0	6.0
1	0.066	100 0	57 8	0 053	90.5	9 0
2	0 037	100.0	78 0	0.064	97.0	33.0
4	0.042	100 0	91 0	0 045	100.0	54.5
A.O.C.S.* 4%	0.076	97.0	52 0	0 138	90.0	12.0
6	0.093	100.0	91.8	0.075	96.5	59.0
8	0.101	100 0	92 0	0.103	97.5	62 0
10	0.132	100 0	95.5	0 200	99.9	64.0
12	0.241	100 0	93 5	0.141	98.5	66.5

* *American Oil Chemists Society Standard Fuller's earth.*

TABLE X

MISCELLANEOUS BLEACHING EXPERIMENTS ON RAPESEED AND MUSTARD SEED OILS

No.	Description of bleaching and oils used	F.F.A., %	Relative transmission	
			660 mμ	440 mμ
1	Alkali treated rapeseed oil bleached for 20 min. at 100° C. with 4% Superfiltrol and 1% Darco carbon.	0 56	93.0	61.5
2	Alkali treated rapeseed oil bleached for 20 min. at 100° C. with 4% Superfiltrol, filtered, and then subjected to a similar treatment with 1% Darco carbon.	0 20	81 0	53.0
3	Crude rapeseed oil heated for 5 min. at 100° C. then 4% Superfiltrol added and held at 100° C. for 20 min.	0 29	90 0	7.5
4	Crude rapeseed oil, 1% water added and then treated as above (3).	0.26	91.5	14.1
5	Crude rapeseed oil, 2% water added and then treated as above (3).	0 25	95.2	8 0
6	Crude mustard oil heated for 20 min. at 100° C. with 2% Superfiltrol added, filtered, and operation repeated with another 2% Superfiltrol.	0.55	67.8	5.5
7	Alkali treated mustard oil, and as in (6).	0.27	85.4	19 8

Effects of Oil Refining

Neither crude mustard nor crude rapeseed oil showed satisfactory bleaching unless high percentages of clay were used in conjunction with temperatures substantially higher than 100° C. (Tables IV, V, VI, VII). Conversely, lecithin-free, alkali treated, or alkali refined oils bleached fairly effectively. However, conventional alkali refining yielded bleached oils with substantially better transmission for a given percentage of bleaching clay (Tables VII and IX).

Changes in Transmission of Bleached Oil on Storage for Eight Weeks

The data of Table XI give the relative transmissions of bleached rape and mustard oils as determined initially and after standing in the diffuse light of the laboratory for an eight week period. Aging tended to improve transmission slightly, lecithin-free rape oil showing somewhat greater change than either alkali treated rape or mustard oils.

TABLE XI

EFFECT OF EXPOSURE TO DIFFUSE LIGHT FOR EIGHT WEEKS ON RELATIVE LIGHT
TRANSMISSION OF BLEACHED OILS

(Oils bleached for 20 min. at 100° C.)

Description of oil	Superfiltrol, %	Relative transmission			
		660 mμ		440 mμ	
		Initial	After eight weeks	Initial	After eight weeks
Lecithin-free rape	1	88.7	97 2	8.3	10 5
	2	94.8	100.0	29 1	32 4
	4	96 1	100.0	60 0	64.8
Alkali treated rape	2	92 5	93.0	31.0	34 8
	6	98.0	99 5	71.0	75 5
Alkali treated mustard	2	76.1	80 0	7 0	8.0
	4	91.7	91 5	20.5	22 0
	6	94.1	96.5	34.4	37.0
	10	97.9	99.5	55.9	57.0

Discussion

These results demonstrate that dark, unattractive erucic acid oils from commercial rapeseed or mustard seed screenings are readily amenable to bleaching. As little as 2% Superfiltrol bleaching clay decolorizes alkali refined oils and yields products similar in color to commercial salad oils, such as corn and cottonseed oils. While the most efficient bleaching was done on conventionally alkali refined material, both the lecithin-free and alkali treated oils yielded reasonably satisfactory products. Interest in these alternative refining procedures resulted from a study of German wartime practice (3).

Such modifications in refining may have a bearing on resistance to oxidation and flavor reversion, and may also provide less expensive procedures in the preparation of oils for industrial purposes.

Crude oils could be bleached with fairly good results at temperatures of the order of 200° C. (Table VII) but an appreciable increase in free fatty acid content occurred. German technology indicates that such oils with 2% or less free fatty acid content may be deslimed with spent bleaching clay, hydrogenated, and then alkali refined. Table V shows that fairly good bleaching of crude oil occurred with 4% bleaching clay and temperatures between 175° and 200° C. with only a comparatively slight increase in the free fatty acid content (0.6%). These results suggest the possibility of applying the German practice. It is possible that direct bleaching of these dark oils at a relatively high temperature may be an economic procedure preparatory to the preparation of sulphated, thermally polymerized, or other industrial oils.

Acknowledgment

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CANADIAN ERUCIC ACID OILS

II. EDIBLE USE OF RAPE AND MUSTARD SEED OILS

By H. J. Lips, N. H. Grace and Elinor M. Hamilton

CANADIAN ERUCIC ACID OILS

II. EDIBLE USE OF RAPE AND MUSTARD SEED OILS[1]

BY H. J. LIPS[2], N. H. GRACE[2] AND ELINOR M. HAMILTON[3]

Abstract

Canadian grown rape and mustard seed oils were alkali refined with 10° Bé. sodium hydroxide, bleached for 20 min. at 212° F. with 2 or 4% Superfiltrol, and deodorized at 464° F. for one hour. These oils were lighter in color and more viscous than commercial corn oil, had smoke points over 400° F., and were clear at refrigerator temperature (40° F.). Consumer acceptance tests indicated that the erucic acid oils, either fresh or aged for 10 days at 100° F., were generally as acceptable as corn oil when used as salad oils or for the preparation of mayonnaise and pastry. The fresh oils were generally not as satisfactory as corn oil for the preparation of doughnuts, although some of the aged oils gave results comparable with those for aged corn oil.

Introduction

The possibilities of Canadian-grown erucic acid oils as edible fat sources were suggested in an earlier publication (6). Cold pressed rape and mustard oils have relatively mild flavor and odor and have long been used for edible purposes, chiefly in the Orient and some European countries, but the hot pressed or solvent extracted oils have strong odor and flavor and are not palatable in the unprocessed state. Improved materials of the latter type have had little culinary application except in Germany (6), perhaps because of anticipation of difficulties with flavor reversion (3, p. 194).

The chief requirements for a good salad and cooking oil are: attractive color, agreeable "feel" or "texture" to the palate, bland or pleasing flavor, good keeping quality, high smoke point (preferably over 400° F.), and clarity at refrigerator temperature (40° F.). These points are considered in the present paper in regard to hot pressed, fully refined rape and mustard seed oils, as indications of their acceptability for food use.

Materials and Methods

The crude rape and mustard seed oils used in this experiment were described in the first paper of the series (5). They were refined with 10° Bé. alkali, bleached with 2 or 4% Superfiltrol under carbon dioxide for 20 min. at 212° F., and steam deodorized under vacuum at 464° F. for one hour in the laboratory according to accepted processing methods (3). A special refined, 4% bleached rape oil was prepared by the use of water washing techniques, before and after refining, to remove phosphatides. This procedure was described in

reports on the German fat industry as an effective means of eliminating flavor reversion (6). The resulting oil contained no detectable quantity of phosphorus and hence was termed "lecithin-free." Each test sample of processed erucic acid oil was a composite of either two or three batches individually prepared from the same source material. Commercially processed corn (salad) oil was used as a reference material in all tests, and comparisons were made on fresh oils and oils aged for 10 days at 100° F. None of the oils showed clouding or precipitation when held at 40° F.

Measurements of color (5), fluorescence (7), kinematic viscosity (2, p. 647), peroxide oxygen (4), free fatty acid (9), and smoke point (1) were made at different stages of processing and use.

Mayonnaise, pastry, and doughnuts were prepared according to the recipes given in Table I. Pastry was baked 10 min. at 435° F. and served at room temperature, and doughnuts were fried in the oils at 355° to 365° F. for three minutes and served warm.

TABLE I

RECIPES FOR VEGETABLE OIL PRODUCTS, GIVEN IN GRAMS

Constituent	Product		
	Mayonnaise	Pastry	Doughnuts
Eggs	—	—	96
Egg yolk	18	—	—
Flour	—	112	500
Sugar	2½	—	200
Oil	200	50	—
Shortening	—	—	25
Milk	—	—	244
Water	15	15-20	—
Baking powder	—	—	15
Cinnamon	—	—	½
Mustard	½	—	—
Nutmeg	—	—	½
Paprika	Trace	—	—
Pepper	Trace	—	—
Salt	1	2	4
Vinegar	15	—	—

The oils and prepared products were scored by a 24-member panel (8); zero represented the ideal in the estimation of the scorer, and excess or deficiency of the property under study was rated on an integral scale of + 5 to − 5. A score numerically greater than ± 2.5 units indicated a definitely unacceptable product. The oils and mayonnaises were rated for color, texture, odor, and flavor, but the pastries and doughnuts were rated for odor and flavor only. All organoleptic data were examined statistically by means of analyses of variance.

Results

Physical and chemical measurements for the corn, mustard, and rapeseed oils at various stages of processing and use are given in Table II. Tables III and IV show organoleptic data for the oils and for mayonnaise, pastry, and doughnuts prepared with them.

TABLE II

CHEMICAL AND PHYSICAL MEASUREMENTS ON VEGETABLE OILS AT VARIOUS STAGES OF PROCESSING AND USE

Oil	Condition of oil				
	1 Bleached	2 Bleached and deodorized	3 Bleached, deodorized, and aged	4 As in column 2, fried	5 As in column 3, fried
	Measurement				
	A. Transmission, %, at 440 mμ relative to mineral oil (Stanolax)				
Corn*	—	32	25	28	27
Mustard, 2% bleach	38	58	51	43	43
Mustard, 4% bleach	50	62	54	44	43
Rape, 2% bleach	75	93	84	64	60
Rape, 4% bleach	85	89	87	62	59
Rape, "lecithin-free"	82	87	84	—	57
	B. Fluorescence, Coleman photofluorometer units, 1 gm. of oil in 100 ml. xylol, corrected for fluorescence of xylol				
Corn*	—	71	67	50	49
Mustard, 2% bleach	70	58	57	50	49
Mustard, 4% bleach	68	56	57	58	47
Rape, 2% bleach	11	2	6	10	11
Rape, 4% bleach	3	2	2	10	12
Rape, "lecithin-free"	16	12	11	—	17
	C. Viscosity, centistokes at 100° F.				
Corn*	—	33.6	33.5	35.7	34.7
Mustard, 2% bleach	41.6	42.3	42.3	44.6	44.9
Mustard, 4% bleach	41.9	42.3	42.5	44.4	44.4
Rape, 2% bleach	46.5	47.4	47.6	48.9	49.5
Rape, 4% bleach	46.7	46.8	46.8	48.3	49.1
Rape, "lecithin-free"	46.8	47.6	47.6	—	49.8
	D. Peroxide value, ml. of 0.002 N thiosulphate per gm.				
Corn*	—	0.0	0.0	3.9	4.5
Mustard, 2% bleach	0.0	0.0	0.0	4.9	5.4
Mustard, 4% bleach	0.0	0.0	4.0	4.3	4.6
Rape, 2% bleach	0.0	0.0	2.6	4.5	4.7
Rape, 4% bleach	0.0	0.0	6.0	4.0	4.6
Rape, "lecithin-free"	0.0	0.0	6.2	—	6.1

* *The corn oil was used as purchased and received no further processing. Column 2 gives values for the untreated corn oil and Column 3 for the untreated, aged corn oil.*

TABLE II—*Concluded*

CHEMICAL AND PHYSICAL MEASUREMENTS ON VEGETABLE OILS AT VARIOUS
STAGES OF PROCESSING AND USE—*Concluded*

Oil	Condition of oil				
	1 Bleached	2 Bleached and deodorized	3 Bleached, deodorized, and aged	4 As in column 2, fried	5 As in column 3, fried
	Measurement				
	E. Free fatty acid content, as % oleic acid				
Corn*	—	—	—	0.1	0.1
Mustard, 2% bleach	—	—	—	0.1	0.2
Mustard, 4% bleach	—	—	—	0.2	0.2
Rape, 2% bleach	—	—	—	0.1	0.2
Rape, 4% bleach	—	—	—	0.2	0.2
Rape, "lecithin-free"	—	—	—	—	0.2
	F. Smoke point, ° F.				
Corn*	—	445	—	—	—
Mustard, 2% bleach	—	405	—	—	—
Mustard, 4% bleach	—	405	—	—	—
Rape, 2% bleach	—	420	—	—	—
Rape, 4% bleach	—	425	—	—	—

* *The corn oil was used as purchased and received no further processing. Column 2 gives values for the untreated corn oil and Column 3 for the untreated, aged corn oil.*

TABLE III

DIFFERENCES IN ORGANOLEPTIC RATINGS FOR VEGETABLE OILS AND THEIR PRODUCTS

Material	Texture	Color	Odor	Flavor
Oils	o	**	o	**
Mayonnaise	o	**	o	o
Pastry	—	—	o	**
Doughnuts	—	—	**	**

o *Indicates no significant differences found.*

** *Indicates highly significant differences (1% level); these differences are shown in detail in Table IV.*

The three oils exhibited differences in both experimental measurements and organoleptic ratings. The relative light transmission of the oils at 440 mμ increased considerably in the order: corn, mustard, and rape (Table II), and the scoring panel was able to detect significant differences in color of oils and mayonnaises (Table III). Relative transmissions for the oils at 660 mμ were all in the range 97 to 100%, so these are not reported. Although mustard oil appeared to be the most fluorescent in daylight, corn oil had the greatest fluorescence in ultraviolet light (375 mμ) with mustard intermediate, and rape least. "Lecithin-free" rape was more fluorescent in ultraviolet light

TABLE IV

ORGANOLEPTIC RATINGS FOR VEGETABLE OILS AND THEIR PRODUCTS FOR THOSE
ATTRIBUTES THAT SHOWED SIGNIFICANT DIFFERENCES

| | Oil | | | | Mayonnaise | | Pastry | | Doughnuts | | | |
| | Color | | Flavor | | Color | | Flavor | | Odor | | Flavor | |
	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged
Corn oil	+0.8	+1.0	+0.9	+0.1	+0.3	+0.1	+1.2	+0.3	+0.1	+0.3	+0.1	+0.5
Mustard, 2% bleach	−0.2	+0.1	+0.1	+0.1	−0.6	−0.1	+0.9	+0.7	+0.8	+0 6	+1.4	+1.0
Mustard, 4% bleach	−0.4	−0.1	−0.3	+0.8	−0.1	−0.5	+0.6	+1.4	+1.4	+0.8	+1.5	+0.8
Rape, 2% bleach	−1.6	−1.7	−0.5	0.0	−0.8	−0.6	+1.0	+0.5	+0.4	+0.4	+0.9	+0.4
Rape, 4% bleach	−1.4	−2.0	−0.1	+1.0	−0.6	−0.7	+0.7	+1.1	+0.5	+1.6	+1.0	+2.3
Rape, "lecithin-free"		−1.7		−0.3		−0.9		+1.6		+0.5		+0.6
Necessary difference (5% level)	±0.5		±0.6		±0.4		±0.5		±0.4		±0.5	

than the other rape oils. Corn, mustard, and rape oils showed increasing viscosity in that sequence, but the panel was unable to detect significant differences in texture of oils or mayonnaises. All initial peroxide values were nil; free fatty acid content of oils after use for frying doughnuts was uniformly small; and smoke points were high, with a slight increase from mustard to rape to corn oil. In spite of the apparent similarity in quality, as assessed by these objective methods, the scoring panel was able to detect significant differences in flavor and odor in some of the products (Table III).

The characteristics of the oils were altered by processing, aging, and frying (Table II). Deodorization generally reduced color and fluorescence but increased viscosity slightly; aging intensified color, had little effect on fluorescence, and none on viscosity; frying increased color and viscosity, decreased fluorescence for corn and mustard, and increased fluorescence for rape oil. Peroxide values of aged and fried oils remained quite low; corn and the 2% bleached erucic acid oils showed the least change.

As shown in Table IV, corn oil was considered too dark and rape oil too light, with mustard approaching the ideal. For mayonnaise, color differences were smaller, and only the rape oil products were thought to be deficient in this respect. Flavor scores for the oils deviated significantly from the nil value for fresh corn oil; and for aged, 4% bleached rape and mustard oils. Pastry and doughnut mean scores were all positive, indicating degrees of excess flavor and odor attributable to the oils used. The erucic acid oils were not as satisfactory as corn oil for the preparation of doughnuts, although some of the aged oils gave reasonably satisfactory results.

The relatively poor flavor scores for fresh corn oil and for pastry prepared with it indicated that the natural flavor of the oil was considered too strong by some of the tasters. This flavor was evidently diminished or obscured by aging and baking or frying. The apparent improvement on aging of some of

the other oils used in the preparation of pastry and doughnuts may have been due to partial volatilization or breakdown of products of aging when the oils were subjected to baking or frying conditions (3, p. 290).

When processing steps are considered in conjunction with experimental results for the single samples used here (Tables II and IV), it would appear that increasing the bleach for rape and mustard oils from 2 to 4% was not advantageous. There was no important decrease in color and more peroxide was formed on storage in the 4% bleached oils. The specially treated "lecithin-free" rape oil (4% bleach) did not show any superiority in organoleptic scores as compared to 2% bleached rape, but was generally better than 4% bleached rape.

Erucic acid oils have hitherto been used for industrial purposes in Canada. These preliminary results indicate that they also merit serious consideration as possible sources of edible materials.

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Chromatographic Analysis of the Unsaponifiable Matter of Marine Animal Oils*

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ABSTRACT

A method of separation of the unsaponifiable matter of marine animal oils has been developed, using the technique of flowing chromatography. Four fractions were obtained by the successive use of light petroleum, methylene chloride, ethyl ether, and methanol with a column of alumina. On the basis of these studies, the hypothesis is proposed that the substances occurring in any one fraction are of similar chemical structure.

The fatty acid composition of natural fats and fatty oils has long been studied and in recent years notably by Hilditch (1940) and co-workers, among many others. The composition of the unsaponifiable matter ("unsap") which accompanies the triglycerides has received less attention, probably because the amount of unsap in fat is usually small. However, in some marine animal oils there may be a considerable amount of unsap. In some oils the presence of certain components, notably vitamins A and D, in the unsap is of great significance commercially.

Hydrocarbons are present in traces in many marine animal oils and constitute a very considerable portion of the oil in some cases. One or more monohydroxy alcohols are universally present, either esterified or in the free state. Cholesterol, vitamins A and D, some pigments, and fatty alcohols fall in this class. Dihydroxy alcohols, as represented by the glyceryl ethers, form a major proportion of the unsap of some oils. A method of chromatographic analysis is presented for the separation of these groups from the non-saponifiable fraction of marine animal oils.

Many naturally occurring mixtures have been examined by this method. They are discussed in several recent books (Zechmeister and Cholnoky 1941, Strain 1942). The unsaps of fatty oils are among those natural mixtures to have received attention. In some cases the separation of a valuable component was the reason for the investigation as, for instance, vitamin A (Karrer *et al.* 1931, Heilbron *et al.* 1932, Holmes *et al.* 1932-33), and vitamin D (Brockmann 1936). Chromatography has been frequently advocated as a means of separating a vitamin from interfering substances in preparation for its determination. Vitamin A in egg yolk has been separated from xanthophylls (Mann 1943). Vitamin D has been separated from vitamin A and pigments (Marcussen 1939; Ewing *et al.* 1943;

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De Witt and Sullivan 1946). The separation of pigments in intensely coloured marine oils has been accomplished frequently (Burkhardt *et al.* 1934; Bailey 1937, 1938; Mori and Sato 1939). The separation of pigments from plant sources has been described many times (Strain 1942). In addition, as contributions to knowledge, separation of the unsaps of various marine oils has been attempted (Channon *et al.* 1934; Thorbjarnarson *et al.* 1935; Nakamiya 1939; Ruiz 1942).

In some of the investigations mentioned above, the classical method of Tswett was used. A solution of the mixture under study was passed through a vertical column of powdered adsorbent which, after development with more solvent, was cut into portions either empirically or by appearance when coloured bands were present. The separated portions of the column was then extracted with an appropriate solvent and the extracts were analyzed.

In other studies the later method of the "flowing chromatogram" was used. After development of the column with a suitable solvent, another solvent was passed through the column which eluted only the lower band(s) of adsorbed material, resulting in a separation of the mixture originally added to the column. Little or no hint was given as to the basis of selection of suitable solvents. Trappe (1940) gave the clue to this selection. He listed a series of solvents in what he termed an "elutropic series", wherein each stated solvent had a stronger eluting action than any following it regardless of the adsorbent in use. This list simplified the selection of suitable solvents for a flowing chromatogram. A recent paper by Strain (1946) states that the selection is not always so simple as indicated by Trappe.

EXPERIMENTAL

The first problem attacked in this investigation was the selection of an adsorbent suitable for the adsorption of vitamin A. Solutions of fish liver oil unsap containing vitamin A were made up in light petroleum and were passed through columns, each containing a different one of seventy-one powdered chemicals taken directly from their containers. Passage of vitamin A through the column into the eluate was followed by examination with ultraviolet light, in which vitamin A exhibited a characteristic greenish-yellow fluorescence. Successive portions of the eluates were examined for their vitamin A content by the Carr-Price reaction, using a Rosenheim-Schüster tintometer. (An Evelyn photoelectric colorimeter was used in all subsequent determinations.) Most of the chemicals allowed unimpeded passage of vitamin A, the initial eluate showing the same concentration of vitamin as the original solution. A few caused marked destruction of the vitamin, and several showed favourable adsorptive properties. The detailed results of this preliminary investigation have been published (Swain 1941).

Since alumina was found to be satisfactory and was readily available commercially, it was selected as the adsorbent for the present studies. In the earlier work to be described below, 40- to 80-mesh activated alumina (Aluminum Ore Co. of America, Grade A) was ground to pass an 80-mesh sieve, and was used without further treatment. Pressure from a cylinder of nitrogen was necessary

to force solvents through the column. In later work grade F1 alumina from the same source was ground to pass an 80-mesh sieve and the portion retained by a 150-mesh sieve was used. This portion was heated at 180°C. for several hours before using. Solvents passed readily through columns of such alumina at atmospheric pressure. In both cases the alumina was packed firmly with a glass rod to a depth of 10 cm. in a glass tube 1 cm. inside diameter. A plug of cotton at the constricted lower end prevented loss of alumina from the column.

Dogfish (*Squalus suckleyi*) liver oil, a valuable source of vitamin A during the war years, was used to find a series of solvents suitable for the separation of the components of fish oil unsaponifiable matter. The unsap was obtained from the oil by the S.P.A. method (Society of Public Analysts Committee 1933), but using four extractions with ether instead of three (Swain 1944). This unsap was dissolved in light petroleum (Skellysolve F) and passed through the column (alumina A, -80 mesh), which was developed by passage of a further 100 ml. of solvent. A yellow band of pigment was usually about two-thirds down the column and did not move during the latter part of the development with light petroleum. Examination with ultraviolet light (G.E. Purple X 250 watt lamp) showed a band of vitamin A fluorescence immediately above the pigment band (figure 1).

In so treating the unsaps of the oils from 30 separate dogfish livers, less than 3% of the unsap was found in the light petroleum eluate in every case. The average amount eluted was 1.7%. The colourless liquid recovered from these eluates gave no precipitate when its acetone solution was saturated with dry hydrogen chloride indicating the absence of squalene (Tsujimoto 1920).

Various solvents and mixtures of solvents were used in an attempt to elute vitamin A from the column without the simultaneous escape of other components of the unsap. Mixtures were unsatisfactory mainly because of the difficulty of maintaining uniformity of composition during recovery for later use. Recourse to solvents in the elutropic series of Trappe (1940) showed that methanol, acetone, ethyl ether and chloroform were all too strong in their eluting ability, since all or most of the unsap escaped from a column washed with one of them. Acetone was useless for another reason also, namely its dimerisation during passage through the column (Zechmeister and Cholnoky 1941, p. 5), resulting in adulteration of the unsap recovered from it. Cyclohexane, a weak eluting solvent, was used to follow light petroleum in the elution of an unsap, but 500 ml. removed only a trace of material from the column.

Benzene (CP) eluted the vitamin A and the pigment, which showed a yellow-green and a reddish-brown fluorescence respectively in ultraviolet light. Progress of vitamin A through the column was determined by measuring the amount present in successive 50-ml. portions of the benzene eluate. Results are shown in table I in which is also given the weight of unsap eluted in each eluate portion. Passage of vitamin A paralleled passage of other components, all dwindling to negligible proportions. This benzene eluate also contained all the cholesterol in the unsap as was demonstrated in the following experiment. One half of a solu-

tion of unsap in light petroleum was passed through a column which was developed with more of the same solvent. Benzene (225 ml.) was then passed through the column. Cholesterol was determined in the benzene eluate and in the other half of the unsap by Ireland's modification (1941) of the Liebermann-Burchard reaction, using an Evelyn photoelectric colorimeter with 540-mμ filter and omitting the preliminary purification procedure. The unsap portion (328.5 mg.) con-
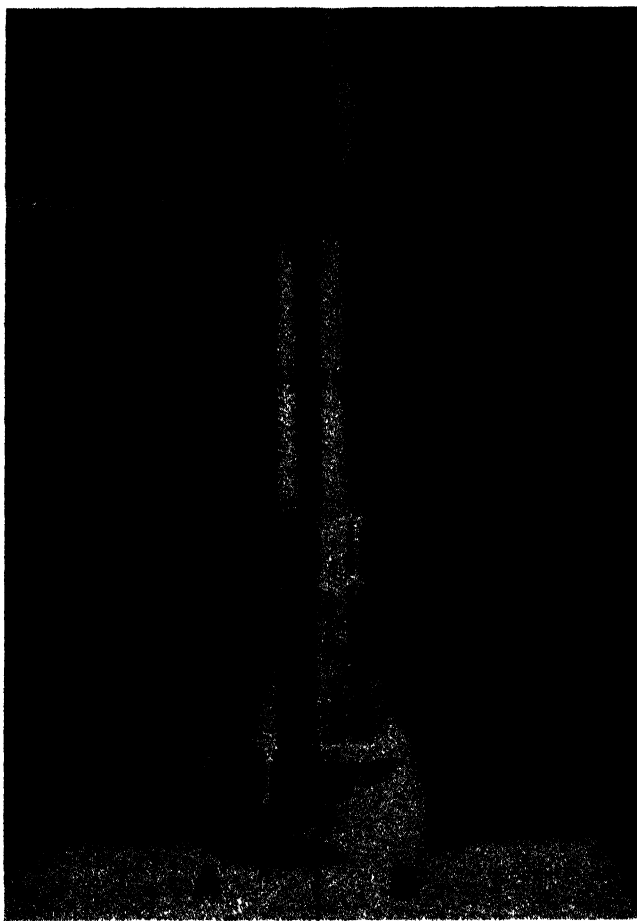

FIGURE 1. Alumina column containing dogfish liver oil unsap, after development with light petroleum. A. Photographed in electric light. B. Photographed in ultraviolet light.

tained 4.32 mg. cholesterol and the benzene eluate residue (45.3 mg.) contained 4.25 mg. cholesterol. That is, essentially all the cholesterol present appeared in the benzene eluate.

Benzene seemed to be a satisfactory solvent, although a rather large volume was required to elute all of the vitamin A. The addition of 2% of ether to benzene increased its power of elution but was not used for long because of the

above-mentioned difficulty of recovery of mixed solvent. Benzene eluted an average of 10.7% (range 3.7 to 28.6%) of unsaps obtained from the thirty dogfish liver oils mentioned above. The material eluted by this solvent contained pigment, vitamin A, cholesterol, and an unidentified liquid.

In an attempt to separate further these substances the residues from several benzene eluates were combined and passed through a column in light petroleum

TABLE I. Vitamin A content of successive benzene eluates from a dogfish liver oil unsap adsorbed on alumina (Alorco, grade A)

Eluate (50-ml. portions)	Residue (mg.)	Vitamin A (U.S.P. units)
1	1.7	80*
2	1.1	30*
3	0.9	40*
4	12.5	262
5	8.7	236
6	3.3	54
7	1.0	23
8	0.3	10

*Approximate values only.

solution. All the material was adsorbed. After development, the column was washed with carbon tetrachloride, an eluant weaker than benzene (Trappe 1940). The solvent was collected in 50-ml. portions in each of which was determined the total weight of dissolved material, the weight of cholesterol and the number of U.S.P. units of vitamin A. The results are shown in figure 2. It is evident that the greatest weight of the benzene eluate fraction, a liquid, was the most readily eluted, and that cholesterol was the most firmly adsorbed. However, no clean-cut separation of the components of this mixture was accomplished.

Passage of ethyl ether (U.S.P., stored over acidified ferrous sulphate and distilled before use) through a column which had been eluted with benzene washed out the greatest proportion of the unsap, averaging 79.5% (range 60.3 to 91.4%) of the unsaps of 24 of the above dogfish liver oils. This material was demonstrated to be glyceryl ethers, earlier reported present in the liver oil from dogfish under the name *Squalus wakiyae* (Toyama 1924), which was later stated to be synonymous with *S. sucklii* (Tsujimoto 1935). Chimyl alcohol was crystallized from its ether or light petroleum solution at −10°C. The crystals were readily separated by centrifuging, providing the cooled solution was first well stirred to enable packing of the crystals. Otherwise much of the mother liquor remained trapped between the crystal plates. After several recrystallizations these crystals amounted to 14-23% of the ether eluate in the oils examined. They melted at 61.5-62°C. (chimyl alcohol 62-62.5°C., Knight 1930) and had a hydroxyl value 10.86 by the method of Malm *et al.* (1935) (chimyl alcohol 10.75). The mother liquors from such crystallizations consisted of a yellow liquid, whose bromination in ethyl ether solution at −2°C. yielded from 0.1 to 2.6% of white precipitate

in those from the oils examined (calculated in each case from the weight of the original eluate residue). Transfer of the ether-soluble portion to light petroleum resulted in a second white precipitate of the same order of quantity. The total gain in weight of the mother liquors on bromination corresponded closely to the calculated value of 46.7% for selachyl alcohol. This eluate therefore contained chimyl alcohol, selachyl alcohol and a small proportion of highly unsaturated
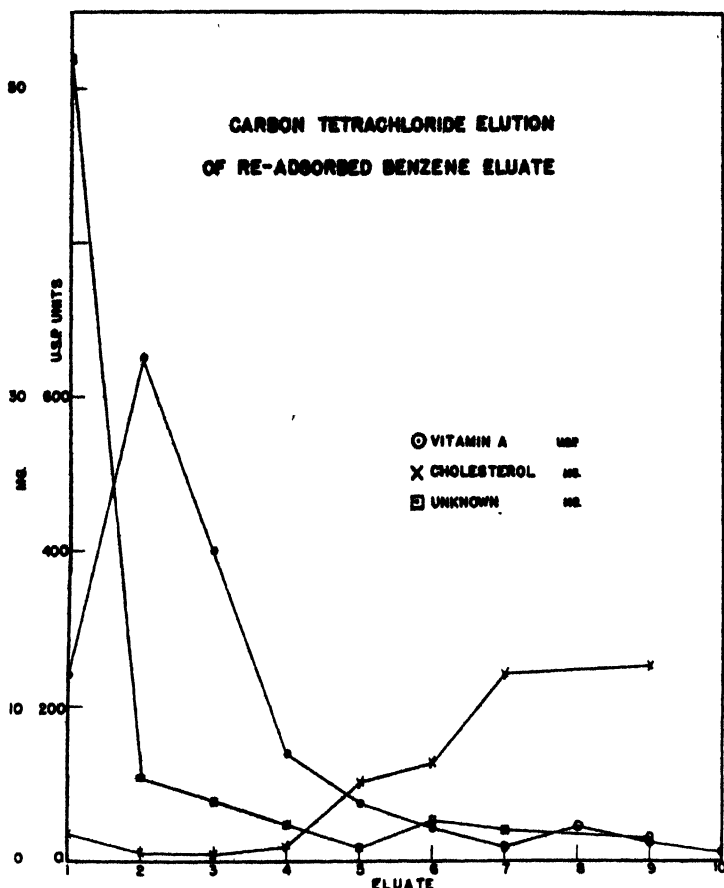

FIGURE 2. Composition of the carbon tetrachloride eluates obtained by chromatographing the benzene eluate residue of a dogfish liver oil unsap, both on alumina.

material, presumably the unsaturated glyceryl ethers reported by Toyama and Takahasi (1939).

Finally, passage of methanol (technical, distilled) through the column eluted 0.5 to 6.1% (average 2.4%) of the unsap of the above dogfish liver oils. This material is unstable. It was liquid when recovered from a column operated in the dark with nitrogen pressure, but semi-solid and only partially soluble in light petroleum when recovered from an unsap passed through a column in the daylight with compressed air.

This series of solvents—light petroleum, benzene, ethyl ether, methanol—thus separated the components of dogfish liver oil unsap into four groups of compounds: (a) A colourless unidentified liquid melting between −6° and 0°C., which was not squalene; (b) pigment, vitamin A, cholesterol, and an unidentified liquid; (c) glyceryl ethers, including chimyl alcohol, selachyl alcohol, and a small proportion of more highly unsaturated material, presumably also glyceryl ethers; (d) a liquid not identified. The absorption curves of these four groups of compounds are shown in figure 3. They were determined on composite samples through the kindness of Dr. G. Halpern of The Canadian Fishing Company, using
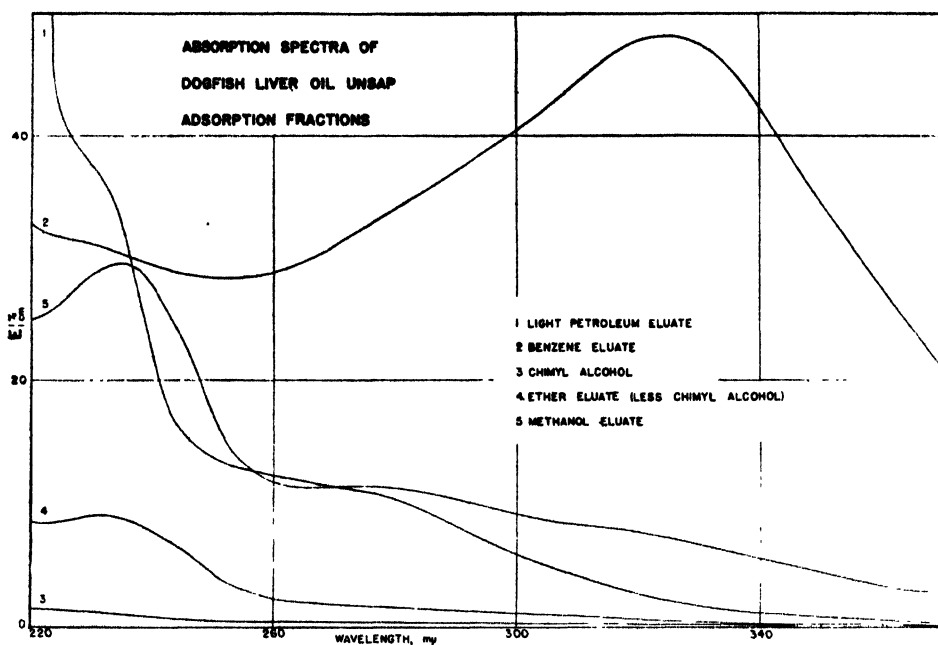
FIGURE 3. Absorption spectra of the eluate residues obtained by chromatographing dogfish liver oil unsap on alumina.

a Beckman D.U. spectrophotometer. With the number of oils examined, no connection was noted between the composition of the unsap and the size or sex of the fish from which it was obtained.

A fresh supply of alumina from the same source, labelled grade F1 but claimed to be identical with grade A, and freshly activated before use, was not as satisfactory as the grade A product (probably because of the activation) in that vitamin A did not escape readily from the column with benzene. Methylene chloride (technical) was found to be sufficiently stronger in eluting ability to remove vitamin A almost completely from the column. Its only disadvantage lay in the fact that it also caused elution of the glyceryl ethers, which were in eluate fractions escaping from the column after most of the vitamin A had been removed. (See table II.)

TABLE II. Fractions separated chromatographically from a dogfish liver oil unsap, using methylene chloride as the eluting agent. C represents cholesterol; F indicates the fluorescence of vitamin A.

Solvent	Volume (ml.)	Eluate residue				Appearance
		Sample 1		Sample 2		
		(mg).	(%)	(mg.)	(%)	
Light petroleum ..	100	2.0	0.4	2.1	0.4	Colourless liquid
Methylene chloride	50	0.9		0.6		Yellow liquid, F
	50	1.0		1.3		" " "
	50	3.2		4.8		" " F, C
	50	11.3		9.6		Yellow solid, F, C
	50	0.2		1.0		C
	50	0	3.0	1.1	3.0	
	50	1.9		5.5		Yellow liquid
	50	18.3		17.8		" "
	50	34.5		46.1		" semi-solid
	50	30.2	15.4	34.0	18.1	" waxy solid
Ethyl ether .	150	323.7		334.0		" solid
	150	14.6	61.7	20.9	61.6	" "
Methanol.... ...	100	25 5	4 7	28 9	5.0	" liquid

The procedure finally adopted was as follows:

Alumina (Alorco grade F1), 80- to 150-mesh size, was used to pack the column. A light petroleum solution of unsap of about 25% concentration was passed into the column, which was developed with light petroleum until the colour band was sharp and without movement during passage of solvent, and until dissolved material no longer was present in the eluate fractions; 150 ml. normally sufficed. Methylene chloride was then added to the column. When it had penetrated to the colour band, the latter appeared red with the ultraviolet light source used, and the receiver was then changed. The solution was collected in 50-ml. portions from which the solvent was removed and the residues weighed. These decreased successively to a very small quantity and then began to increase. The vitamin A fluorescence was very faint in eluates following those containing only a small amount of solute. The increasing weights were due to elution of the glyceryl ethers. Ether was then passed through until 150-ml. portions of the eluate contained little or no residue. This solvent eluted the glyceryl ethers much more readily. Finally, passage of 100 ml. of methanol through the column eluted the remainder of the adsorbed material.

In table III are given the results of three separate analyses of samples of unsap prepared from one oil, showing the replicability of the method. In table IV are the results obtained from samples of commercial dogfish liver oils produced in British Columbia.

TABLE III. Chromatographic analysis of samples of a dogfish liver oil unsap (Alumina F1).
Bracketed values are considered to be glyceryl ethers and are included in the ether eluate totals.

Solvent	Volume (ml.)	1 Eluate residue		2 Eluate residue		3 Eluate residue	
		(mg.)	(%)	(mg.)	(%)	(mg.)	(%)
Light petroleum........	50	7.0		8.1		8.3	
	50	1.3		0.9		0.7	
	50	0.7		0		0.1	
		9.0	2.1	9.0	2.1	9.1	2.0
Methylene chloride.....	100	12.7		15.0		14.9	
	50	16.0		19.2		13.8	
	50	5.0		2.3		6.3	
	50	2.3		0.1		2.0	
		36.0	8.4	36.6	8.6	37.0	8.3
		(14.9)		(13.6)		(11.6)	
Ethyl ether...........	150	285.7		282.1		283.0	
	150	41.8		54.8		50.8	
	50	5.5		1.7		6.2	
	50	3.2		2.8		4.2	
	50	2.9		2.4		2.3	
	50					2.8	
		354.0	82.6	357.4	83.6	360.9	81.3
Methanol.............	100	27.4	6.4	27.2	6.4	26.0	5.9
Recovery....... .			99.5		100.7		97.5

TABLE IV. Chromatographic analysis of the unsaps from commercial British Columbia
dogfish liver oils (Alumina F1)

Oil no.	Unsap (%)	Eluate residue (%)			
		Light pet.	Methylene chloride	Ethyl ether	Methanol
1	27.5	0.6	4.1	78.5	10.8
2	22.2	1.4	7.2	76.3	12.1
3	20.6	0.9	8.3	69.1	12.3
4	25.0	0.8	14.7	87.1	4.4
5	23.8	3.1	11.3	78.6	7.6
6	22.3	1.7	8.9	78.9	5.7
7	20.6	1.7	7.2	79.5	8.8
8	23.2	1.7	5.8	84.1	3.3

During the development of this method of analysis, it was applied to the unsaps of oils from other marine sources (Swain and McKercher 1945). The details of procedure varied with the stage of development of the method. In all cases a given solvent was used until the residue in a given volume of eluate became negligible in amount. The results are shown in table V.

TABLE V. Chromatographic analysis of the unsaps of several marine animal oils.

Unsap from oil of	Alumina	Light petroleum		Second solvent		Ethyl ether		Methanol	
		(ml.)	(% eluted)	(ml.)	(% eluted)	(ml.)	(% eluted)	(ml.)	(% eluted)
Halibut liver	A	150	1.0	2100°	91.8	300	9.3	100	4.9
Lingcod liver.	A	100	1.7	900*	78.6	300	12.3	100	6.5
	A	100	1.6	900*	78.7	300	12.2	100	5.1
	F1	100	1.3	500†	70.0	300	18.8	100	5.7
	F1	100	1.7	500†	69.1	300	17.7	100	7.5
Basking shark liver .	F1	225	98.1	350†	5.4	150	1.3	100	1.6
	F1	225	93 6	350†	4.7	150	1.5	100	1.7
Soup-fin shark liver.	F1	600	2.8	600†	40.8	300	11.9	900	36.3
Mackerel shark liver	F1	200	1.7	550†	77.7	150	4.9	100	12.1
Sperm whale blubber	F1	250	0.7	700†	88.9	150	1.0	100	5.9
Rat-fish liver.	F1	150	1.2	300†	29.7	450	68.6	100	1.7

*2% ether in benzene.   †Methylene chloride.

The unsap from the liver oil of halibut is known to be mainly cholesterol (Haines and Drummond 1933). This was demonstrated with a sample of oil containing 8.5% unsap. The portion of unsap readily soluble in light petroleum was added in that solvent to a column of alumina. After development with the same solvent, the remainder of the sample of unsap was dissolved in benzene and added to the column. Most of the sample was eluted from the column by benzene, and the melting point of the residues from the later fractions showed them to be almost pure cholesterol. The vitamin A in the unsap also appeared in these benzene eluates.

A sample of liver oil from lingcod (*Ophiodon elongatus*) contained 12.2% unsap, a deep yellow liquid in which were suspended a few crystals at room temperature. Chromatographic analysis of the unsap yielded a pale yellow solid in the light petroleum eluate, with an intense band of pigment remaining in the column. Two per cent ether-in-benzene mixture eluted most of the unsap. In a later analysis methylene chloride eluted slightly less of the unsap. In this experiment the solid eluted by light petroleum was tested with the Liebermann-Burchard reagent and was shown to be free from cholesterol.

The unsap of the liver oil from the basking shark (*Cetorhinus maximus*) is known to be largely the hydrocarbon squalene (Tsujimoto 1917; André and Canal

1928). The unsap from a sample of this oil (52.2% of the oil, and a colourless mobile liquid) passed through a column of alumina almost completely in the light petroleum in which it was dissolved. The eluate residue remained liquid at −25°C. Passage of hydrogen chloride through a cold acetone solution of the residue produced a copious white precipitate with m.p. 120-1°C., a test for squalene (Tsujimoto 1920). This experiment offered confirmation of the earlier work and showed that the hydrocarbon squalene passes through the column as rapidly as does the solvent light petroleum, since practically all of it appeared in the first two eluates.

The writer has seen no description of the composition of the unsap of soup-fin shark liver oil (*Galeorhinus galeus*) other than relative to its high content of vitamin A. The oil from a composite sample representing over 100 cans of livers contained 5.7% unsap, a viscous yellow liquid at room temperature. The small portion of it which was eluted by light petroleum was a white solid. The fractions eluted by methylene chloride were solid, probably because of the cholesterol content. All other fractions eluted from the column were liquid, the proportion eluted by methanol being uniquely large among the oils examined.

The liver oil from a mackerel shark (*Isurus nasus*) (Swain and Sidaway 1944) contained 7.9% of an unsap which by chromatographic analysis was quite similar in composition to that of halibut liver oil.

A sample of oil prepared commercially from the blubber of the sperm whale (*Physeter macrocephalus*) led to interesting results. The unsap of this oil is known to consist mainly of fatty alcohols (Toyama 1927), and amounted to 35.8% of the oil examined. Almost none of it was eluted by light petroleum in its chromatographic analysis and no band of pigment was evident. Elution of the column with methylene chloride washed out nearly all the unsap, from which it follows that this fraction must therefore contain the fatty alcohols. The appearance of successive eluates implied that the liquid oleyl alcohol was eluted somewhat ahead of the solid cetyl and stearyl alcohols.

The liver oil from the rat-fish (*Hydrolagus colliei*) is known to contain glyceryl ethers (Lovern 1937). The unsap from a sample of oil, containing 33.6% unsap, gave results on chromatographic analysis similar to those obtained with dogfish liver oil unsap.

These data reduce the universality of a method for the determination of unsap proposed by Sylvester *et al.* (1945). In this method the ether extract prepared by the S.P.A. method is acidified without previous washing. The resulting ether solution is water-washed to remove the inorganic acid and dried. The solution of unsap and free fatty acids is passed through a column of alumina1 which is then washed with more ether. The unsap is claimed to pass through and the free fatty acids to be adsorbed. However, in all the oils reported above ethyl ether did not elute all of the unsap. In the case of mackerel shark liver oil and of soup-fin shark liver oil, a very considerable portion of the unsap remained in the column after elution with this solvent. The unsap as determined by this method will therefore be lacking by that amount.

On the other hand, some free fatty acids are eluted by ethyl ether. Dogfish liver oil fatty acids (0.5 g.) prepared by acidifying the soap solution remaining after extraction of an unsap were passed in light petroleum solution through a column of alumina which was then developed with more light petroleum until 150 ml. eluate was obtained. This eluate left no residue on evaporation. Passage of 150 ml. methylene chloride eluted 0.8% of the fatty acids, and subsequent passage of 300 ml. ethyl ether eluted 42.3%. The last 50-ml. portion still contained 5% of the total material eluted. The unsap as determined by the method of Sylvester *et al.* may therefore contain free fatty acids, and may not include all the components of the unsap of the oil.

DISCUSSION

The results obtained in this series of experiments suggest that substances of similar chemical structure are eluted by a given solvent, with some partial degree of separation because of variations of that chemical structure. It is recognized that hydrocarbons are eluted by light petroleum (Fitelson 1943). This was demonstrated with the unsap from basking shark liver oil, from which squalene was shown to be separated in a column by light petroleum.

The substances recognized to be present in the methylene chloride eluate included vitamin A, cholesterol and fatty alcohols. These are all monohydroxy in composition. A partial separation of vitamin A and cholesterol was evident by visual examination of the eluate residues together with measurement of the vitamin A. This separation was demonstrated by the use of carbon tetrachloride as eluting solvent, a solvent below methylene chloride in Trappe's series. The alcohols in sperm whale oil unsap were partially separated as evidenced by the difference in solidity of successive eluate residues.

Ethyl ether eluted the glyceryl ethers from the unsaps of dogfish and of ratfish liver oils. Chimyl alcohol and selachyl alcohol were recognized to be present in dogfish liver oil unsap and also material sufficiently unsaturated to be precipitated by bromination in ether solution. In this eluate, compounds of varying C-atom content and of varying unsaturation shared the property of being dihydroxy in nature, which is possibly the dominant characteristic of the ethyl ether eluate.

The material eluted by methanol was in most cases too small in amount for examination. It seemed to be unstable in nature.

SUMMARY

The unsaps of marine animal oils are separable into simpler mixtures of compounds by the method of the flowing chromatogram. It was shown that hydrocarbons such as squalene are not adsorbed from light petroleum by alumina. Of the other substances present, monohydroxy substances are least readily adsorbed being eluted by benzene or, more readily, by methylene chloride; and dihydroxy substances are eluted with difficulty by methylene chloride but readily with ethyl

ether. Other substances, not eluted by ether, are removed from the column by passage of methanol.

The unsap of dogfish liver oil was shown to contain only a very small proportion of hydrocarbons but rather to be largely glyceryl ethers. The composition of the unsaps of oils from individual dogfish livers showed quantitative differences. The unsap of a rat-fish liver oil was of qualitatively similar composition.

The unsaps from oils of other fishes and from the sperm whale were found to differ in composition very considerably. The unsap from basking shark liver oil was largely unadsorbed, and is known to be mainly hydrocarbon in nature; the unsaps from sperm whale oil and halibut liver oil were mostly eluted by benzene or methylene chloride and are known to be principally monohydroxy substances.

A published method for the determination of unsaponifiable matter was shown to be open to two possible errors, namely, incomplete elution of unsap from alumina by ethyl ether, and incomplete adsorption of fatty acids from ethyl ether by alumina.

ACKNOWLEDGMENTS

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BROWN DISCOLORATION IN MALTED PROCESS CHEESE [1]

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Malted cheese is a blend of process cheese with malt syrup. On prolonged storage, without refrigeration, as on the shelves of retail stores, its rich yellow-orange color is gradually changed into a dull orange-brown. The manufacturers sought an explanation of the color deterioration which was objectionable from the viewpoint of merchandising. The flavor of the cheese, however, remained acceptable. Some of the circumstances which were associated with the appearance of the discoloration were the change from an opaque tin-foil to a transparent cellophane wrapper, use of pasteurized malt, and a higher processing temperature of the cheese.

A study of the defect indicated an interaction of the amino acids and proteins of the cheese with aldose sugars in the malt. Frankel and Katchalsky (1937), in studying this type of reaction, showed that it was accompanied by an increase in titratable acidity and a consequent lowering of pH.

Accordingly, comparative packages of malted cheese were stored at 4.4 and 26.7°C.(40 and 80°F.). While at the lower temperature the cheese retained its color, at the higher storage temperature discoloration became perceptible after one month and increased in intensity thereafter. At this time titratable acidities and pH values were determined (Table 1). The titratable acidities are on a per-gram dry-weight basis. It may be readily seen that the titration values are definitely higher and the pH values significantly lower in discolored cheese as compared with normal cheese.

As a further confirmation that the interaction of amino acids with aldoses was the chemical basis of the discoloration, this defect has been successfully reproduced in the laboratory by the addition of chemically pure glucose to ordinary process cheese followed by prolonged storage at 80°F. Also a fluorometric determination on a NaCl extract of acetone-defatted, discolored, malted cheese by the method of Pearce, Thistle, and Reid (1943) gave a higher fluorescence value than for normal malted cheese. Other instances in which the introduction of aldose sugars to milk products resulted in brown discoloration upon heating have been reported by Ramsey, Tracy, and Ruehe (1933) and by Tracy and Edman (1942); and the chemistry of nonenzymatic browning has engaged the attention of chemists in several fields, American Chemical Society (1946).

On storing malted cheese which was prepared with raw and pasteurized malt, no appreciable difference in the discoloration was noted. Since malt

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for other purposes is now generally pasteurized, this product is used for the manufacture of malted cheese. A higher processing temperature was temporarily adopted because of a shortage of suitable wrapper coating during the war. Experiments revealed a slightly greater rate of color deterioration in cheese processed at the higher temperature. Since then, suitable wrapper coating has made it possible to return to the original processing temperature. Nevertheless, the defect, actual or potential, still exists although it has been overcome to some extent by more frequent servicing of retail stores. The cellophane wrapper, although more revealing, has now been adopted because of its greater eye appeal.

TABLE 1

Titration and pH Values of Normal and Discolored Malted Cheese After Four Months' Storage

No.	Normal (stored at 40°F.)		Discolored (stored at 80°F.)	
	ml. $\frac{N}{10}$ NaOH	pH	ml. $\frac{N}{10}$ NaOH	pH
1	4.7	5.88	8.7	5.72
2	4.0	6.00	7.1	5.67
3	4.4	5.98	7.4	5.65
4	4.4	5.98	7.4	5.73

Webb (1935) and Kaufman (1946) have shown sulfur dioxide to be effective in preventing the browning reaction. Whether this is due to the well-known property of bisulfite of combining with aldehyde groups and thus blocking the reaction of aldoses with amino compounds has not been established but is an interesting possibility.

Sodium bisulfite calculated to give a concentration of 500 p.p.m. SO_2 was added to a commercial batch of malted cheese. When initially assayed by the Monier-Williams method, Association of Official Agricultural Chemists (1945), it was found to contain 160 p.p.m. SO_2. Regular half-pound packages of normal and sulfited malted cheese were then stored at 40 and 80°F. for a period of 10 weeks. At 80°F. good color was retained in the treated cheese throughout the storage period. The SO_2-free comparative samples, however, showed a pronounced discoloration at the end of five weeks. Analysis of the treated cheese at the end of the trial period showed practically no decrease in the SO_2 concentration at 40°F., but a decrease to 58 p.p.m. at 80°F. The cheese held at 80°F. was, therefore, still protected against discoloration in spite of the favorable conditions for accelerated deterioration. It is reasonable to conclude, then, that under less drastic temperature conditions in the usual retail channels malted process cheese can be protected from discoloration by the addition of sulfites or sulfur dioxide.

The use of sulfur dioxide in food products is, of course, regulated by the various departments of public health. Proper cognizance should, therefore, be taken of the appropriate regulations should commercial application of the above scientific findings be contemplated.

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POSSIBILITIES IN DEVELOPING FISHERIES BY-PRODUCTS[a]

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REVIEW PAPER

The author reviews some of the phases of fishery by-products in the light of the more recent work, particularly fish meal, recovery and uses of "stick-water," proteins, fish wastes and possibilities of obtaining biological products from these wastes.

It will soon be twenty-five years since D. K. Tressler published, in collaboration with others, a book which I think has proven invaluable to most fishery technologists *(98)*. This book was entitled "Marine Products of Commerce," and a new edition has recently been published. It seems unfortunate that this book has not been revised in order to make it thoroughly up-to-date, though there is no doubt that some of the sections could pass with little revision. In this work, a number of chapters are devoted to by-products of the world's fisheries, and a perusal of these is sufficient to assure one that a vast variety of such products exists. In the time at my disposal it will be possible to cover only some of the phases of fishery by-products research, and in most instances no attempt will be made to state whether commercial production of any of the various products discussed is likely to prove profitable.

In considering the term "by-product" as related to the fishing industry I have avoided, with a few minor exceptions, reference to any item which is intended for consumption as a nutritious human food. In fact this would perhaps be a useful definition of the above term, since it would automatically exclude such items as fish sausages, loaves, or canned fish trimmings, but could include agar, alginic acid and vitamin oils, which, though consumed, can hardly be considered nutritious foods. It is almost impossible to divide by-products into very strict classes since they are of such varied nature, so I have only attempted to classify them into rough groups. Other reviews dealing with certain by-products are available *(59, 60, 98)*.

BY-PRODUCTS OF A PROTEIN NATURE

The days when, as one fish foreman stated, it was possible to "sell anything with a fin on it," seem to be drawing to a close. Meat and other animal protein foods appear to be in more plentiful supply, and it is inevitable that under these conditions a surplus of fish may develop. Indeed, even at the present time, the market for certain of the inferior grades of white fish is becoming restricted on this continent. It is hoped that some of the surplus may be utilized by canning, and a fairly palatable canned product can be prepared if a method which avoids accumulation of surplus liquor in the can, and brown discoloration of the fish is followed *(19)*. However, there is much loss in canning certain types of white fish, and there may be only a twenty percent yield of edible fish in some cases. This inevitably makes cost of production quite high and the product must compete with tasty canned fish such as the higher grades of salmon, sardines, etc. I think then, that the most important single problem which will require solution is that of disposal of surplus first class fish protein at a price which is profitable to fishermen and to the industry. There is plenty of room for research in this field.

The chief protein by-product of the fishing industry is fish meal which is normally produced from so-called "scrap fish," fish offal and shell fish wastes. The general methods for commercial production of fish meal are quite well-known and will not be discussed *(21, 59)*. One west coast firm is drying the cooked flesh by

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passing hot air through a thin layer of the comminuted material in order to avoid undue heating of the product which is liable to occur by ordinary drying methods. Fish meals, especially those containing fish liver and "solubles" *(11, 57)*, are usually in great demand for addition to livestock rations, for they generally contain adequate concentrations of the essential amino acids *(1)*. However, the raw material used in their preparation may occasionally contain much poorly digestible cartilaginous material, or perhaps urea, and it has been suggested that in using meals some cognizance should be taken of their content of amino acids essential for livestock feeding rather than simply referring to their nitrogen content. Present methods of determining amino acids are rather time consuming and exacting so it is difficult to decide whether or not the above suggestion is feasible. At our Station a study of the essential amino acid content of certain fishery products is being made using microbiological assay methods *(29)*, and it is intended to extend this work to include a survey of different fish meals and to eventually attempt the isolation of amino acids from fish proteins. High quality fish meal has been said to be one of the few protein sources which will support chick growth entirely by itself *(1)*. Recently it has been established that it contains a "Factor S" which is apparently essential for growing chicks, and that this factor appears to be identical with the Strepogenin recently discovered by Woolley at the Rockefeller Institute *(89)*.

Fish meal is normally prepared by cooking the flesh and pressing it. The cloudy press liquid which results after removing the greater part of the oil by centrifuging, or by use of settling tanks, still contains suspended and soluble proteinaceous material, and some oil. This liquid is commonly known as "stickwater" or fish press liquor, and it has been realized for some time that much valuable nitrogenous material is wasted when this is discarded *(7, 29)*, and that it is liable to cause undesirable pollution of rivers or harbors if it is discarded *(56)*. Attempts have been made to recover the proteinaceous material from these waste liquors *(7, 50)*, but it is apparently only quite recently that recovery has been realized commercially, and even now large quantities are wasted annually in British Columbia. The liquor may be treated in two different ways for incorporation in livestock feeds. At present the best procedure appears to be that of multiple effect evaporation, so that a concentrate containing about 50 percent of solid matter is obtained. This thick liquid can be added directly back to the comminuted cooked flesh before it is dried into meal, incorporated in dry kelp *(Macrocystis)* meal for livestock feeding or may even be shipped in tank cars and incorporated in feeds at its destination. Alternatively it may be fractionated as follows (56) : It is first adjusted to pH 4 to 5 with an acid protein coagulant such as sulfuric or tannic acid. This breaks the emulsion and when the liquor is centrifuged, from 0.5 to 2.0 percent of oil is recovered and the oil alone is said to be of sufficient value to pay for the whole treatment procedure. The protein solids may be filtered off; they account for about 2 to 3 percent of the liquor and may be dried with the fish meal. The clear, sparkling filtrate contains soluble protein material and the B vitamins, thiamine and riboflavin. It may be concentrated by evaporation, or, at its low pH value the B vitamins may be adsorbed on fuller's earth which is added in proportions of 0.5 to 1 percent. The fuller's earth, which contains the adsorbed vitamins, can be dried, after removal by centrifugation, and added directly to livestock feeds. Another suggested method for separation of protein solids from stickwater involves saturation of the hot liquor with a salt such as sodium chloride or ammonium chloride, which causes physical flotation of the small particles, cooling and mechanically removing the jellied supernatant layer *(62)*. This method does not appear very practical for it would involve use of enormous quantities of salts. It is said that in Norway, stickwater is mixed with the partially dried meal, so that the meal after final drying is richer in B vitamins and soluble

extractives. An idea of the potential value of stickwater to the fishing industry can be obtained from figures which have been published *(103)* regarding the sardine or pilchard industry. The estimated annual production in 1945 was, in millions of pounds: canned fish, 150; fish meal, 155; oil, 104 and stickwater, 7. The recent poor pilchard runs would seem to make it even more imperative that stickwater be recovered.

The "condensed fish solubles" obtained by concentrating stickwater have valuable nutritive properties *(11, 27)*. When fed at 12 percent level they were found to produce excellent growth in chickens, but above this level exerted a cathartic action. When both diets were supplemented with riboflavin the press water concentrate was as effective as skim milk in chick nutrition *(57)*.

The value of fish wastes as fertilizers is well-known. In this connection it is interesting to note that incorporation of stickwater into peat moss, which can be subsequently dried, results in an excellent fertilizer which will increase the humus content of poor soils *(62)*. One west coast firm has evolved a method for extracting, from salmon offal or from fatty scrap fish, oils which have a considerably higher vitamin A potency than that normally occurring in oils extracted by usual commercial procedures. The protein residue, which is quite alkaline in reaction, contains large amounts of inorganic salts, and consequently cannot very well be incorporated in livestock rations. However, it has been found to possess valuable properties as a fertilizer. Whether or not it is desirable to increase the yield of vitamin A in the extracted oil at the apparent expense of first-class feeding protein would seem questionable.

It is well-known that certain fish wastes, particularly those containing large amounts of collagen (skin and heads), can be manufactured into glue *(98)*. Fish glue usually contains significant quantities of proteins and peptones and is therefore inclined to be soluble in cold water *(59)*. Dialysis of stickwater to remove salts, and subsequent evaporation, has been found to form a useful method for preparing fish glue *(92, 93)*.

Synthetic egg white prepared from fish protein has been used for some years in Germany *(5, 15, 31, 100)*. The general method of preparation is, apparently, to remove the readily soluble fish flesh protein with dilute acetic acid, and extract fat from the residue with trichlorethylene or ethanol. The extracted residual protein is stirred with warm dilute sodium hydroxide solution. The partially hydrolyzed extracted protein thus obtained is presumably largely myosin. It is neutralized with dilute acetic acid and spray dried to yield a white powder containing 94 percent protein. Its amino acid composition suggests that it would have a high nutritive value.

Fish scales are a source of useful by-products. The scales of several species of fish yield small lustrous crystals of the purine guanine, and these form the pearl essence of commerce *(24, 98)*, which is used to coat the surface of artificial pearls. However, other possibilities have been suggested in the utilization of fish scales. Guanine itself can be made from the scales. It is said that caffein, which chemically is rather closely related to guanine, has been made in considerable quantities from fish scales *(4)*. It is probable that the scales are analogous to human hair in the fact that they apparently contain considerable quantities of cystine; one company is said to use scales as a source of raw material for manufacturing this amino acid.

Certain of the internal organs of fish offer possibilities as sources of hormones, enzymes or other interesting and useful by-products. In 1901 it was shown by Rennie in Scotland that fish contain quite prominent islets of Langerhans, and he thought that these might prove of value in counteracting diabetes. Years later MacLeod, in whose Toronto laboratory Banting, Best and Collip succeeded in

preparing clinically administrable insulin, became interested in the occurrence of insulin in fish. A research program was initiated at the St. Andrews Station of the, then, Biological Board of Canada, and it was found that the principal islet of Langerhans in bony fish is usually attached to the gall bladder, from which it can be separated quite readily. Clinically active insulin, indistinguishable from that prepared from beef pancreas, was extracted from these islets *(65, 66)*. It is difficult to determine whether much insulin has been prepared commercially from fish, though it is said that the Japanese have developed this source. The demand for insulin is increasing so that there are strong possibilities that all available bovine pancreatic tissue may be required for its preparation, *(13)*. If some of the commercial tryptic enzymes were prepared from fish viscera *(vide infra)*, valuable animal pancreatic tissue could be reserved entirely for insulin production. Another pharmaceutical product which has been prepared from fish is the anti-pernicious anaemia factor. Preparation was effected by making a dilute aqueous extract of haddock, cod or whiting livers (pH 4.5), heating it to 158° F. (70° C.), settling, filtering, concentrating in vacuo, precipitating the active principle with 95 percent alcohol and drying to a powder *(61)*. The anti-pernicious anaemia factor so prepared was found to be quite effective in a number of clinical trials *(28)*.

It will be recalled that the classical work of Meischer on the composition of sperm nucleoproteins was carried out over half a century ago, the raw material employed being ripe herring milt. The best known sperm nucleoproteins are clupein, from herring milt, and salmine from salmon milt. Chemically, sperm nucleoproteins consist of nucleic acid and sperm protein, or protamine, which are apparently quite loosely combined, as work by Pollister and Mirsky *(77)* at the Rockefeller Institute has shown. Of chief interest is the fact that these protamines consist of 15 amino acid residues, 10 of which are accounted for as the basic amino acid arginine, and the remainder probably as proline, valine, serine and alanine. Fish milts have been utilized to some extent commercially. Protamine is used to make protamine zinc insulin, which is practically insoluble and when injected liberates insulin slowly, giving a regulated action. Protamine from certain species of Pacific salmon has been employed for this purpose *(2)*. The occurrence of large amounts of arginine in sperm proteins makes the ripe milt of fish a very convenient source of this amino acid. Salmine has been found to reduce the clotting time of blood plasma *(12)*. It has also been found that clupein *(64)*, salmine *(70)*, protamine sulfate from shad *(18)* and protamine zinc insulin *(18)* possess marked antibacterial activity.

One step in the preparation of hide leather involves a softening which is brought about by a slight hydrolysis of collagen and elastin by crude proteolytic enzyme preparations; so-called "leather bates." It has been found that the pyloric caeca of fish (finger-like blind sacs which are attached to the gut just below the stomach, and have a function similar to that of the mammalian pancreas) are a potent source of tryptic enzymes. Crude enzyme preparations made by acetone drying of comminuted caeca were found to be just as effective as hog pancreas or commercial leather bates for leather treatment and for hydrolysis of isolated collagen, elastin and olive oil *(47, 48, 49)*. Recently, finely minced pyloric ceaca have been employed to prepare protein hydrolysates from fish flesh and from fish muscle myosin, and the enzyme preparations used effected about the same degree of hydrolysis as did commercial powdered pancreatin *(29)*. Crystalline salmon pepsin has been prepared from salmon stomachs *(73, 74)* and its specificity has been studied *(33)*. Properties of crude tryptic enzymes from fish viscera have been investigated *(51)*. In our own laboratories attempts are being made to prepare crystalline trypsin-like enzymes from the pyloric caeca of fish.

One suggested outlet for surplus fish flesh protein is that of preparation of plastics of the type which have been commercially made from casein: so-called "artificial horn." There are a number of possible methods whereby such plastics can be prepared. One method which has given good results in the laboratory is to dry comminuted fish flesh with alcohol or acetone at ordinary temperatures. The dry, powdered meal is mixed with 20 percent of ethyl lactate and pressed at about 12,000 lbs. per sq. in. and 203° F. (95° C.), or 10,000 lbs. per sq. in. and 221-239° F. (105-115° C.), for approximately 10 hours. The pressed plastic is then treated for at least 2 weeks in commercial formalin, which hardens it and prevents it from becoming brittle *(45, 46, 52)*. Recently it has been claimed that large sheets of plastic material have been prepared from fish flesh *(4)*.

There are other possible uses for fish flesh proteins. Protein hydrolysates prepared from casein, corn and wheat glutens have been found to be useful as flavoring ingredients for foods *(34)*. Protein hydrolysates are readily prepared from fish muscle, and, superficially at least, do not seem very different from those obtained from meat. This is understandable for, as far as is known, the muscle proteins myosin, myogen, globulin X, collagen and elastin are present in roughly similar proportions in both kinds of muscle *(6, 79)*, though the content in soluble extractives differs *(80)*. Probably it would be of interest to compare hydolysates prepared by tryptic or hydrochloric acid digestion of fish muscle and of cereal proteins with the object of ascertaining whether the former might be of value for addition to foods. In this connection it is of interest that one patent records the preparation of concentrates similar to meat extracts from meat of sea mammals such as whales and seals *(75)*. Possibly, similar extracts could be prepared from fish flesh. Removal of the slight residual "fish" flavor which tends to remain in fish protein preparations intended for use in human foods is said to present rather a difficult problem.

Peptones have been defined as the dried product of the pancreatic digestion of meat *(68)*. They have some commercial use, chiefly in preparation of microbiological media, and it seems reasonable to assume that it would be quite simple, and perhaps cheaper, to prepare them from fish muscle as raw material instead of from meat. A "peptone" has been made by hydrolysis of fish flesh with hydrochloric acid *(102)* and in our laboratories peptones have been prepared by hydrolyzing fish flesh with proteolytic enzymes obtained from fish. In recent years considerable interest has been taken in the so-called "food yeast," *Torulopsis utilis*. This yeast is rich in B vitamins and is comparable to casein in supplementing cereal proteins in a diet since it contains the much needed amino acids lysine and valine. It is somewhat deficient in methionine *(76)*. *T. utilis* grows well on peanut waste liquors containing an added ammonium salt *(55)*, and it seems possible that stickwater might find some similar use as a culture medium. It has apparently been employed in Japan in media used for preparing penicillin.

FATS, FAT SOLUBLE VITAMINS, STEROLS AND PHOSPHOLIPIDS

During the past decade there has been published a very large body of information regarding marine animal fats or oils, their occurrence, characteristics and uses, and much of this has been reviewed *(20, 59, 60)*. The use of hydrogenated marine oils in shortenings, cooking fats and in margarine manufacture is well-known. Fish oils are used and offer possibilities in the paint and varnish industries *(63)*. Other known uses for fish oils include incorporation in linoleums, oilcloths, patent leather, oiled silks, core oils, printing inks, rubber, synthetic detergents, etc.

Fish oils are still the principal source of vitamin A *(85)*, and certain oils such as those of halibut, red cod, ling cod, etc., can be used directly to supply this vitamin clinically. However, the supply of high vitamin A potency oils is usually rather

restricted and considerable effort has been directed toward concentrating vitamin A from fish oils of moderate or low potency, particularly liver oils from dogfish and fish body oils. Molecular distillation can effect a five to fifty times concentration of this vitamin from oils of salt water fish, in which it occurs partly as a free alcohol and partly in the form of its esters *(38)*. Another method of concentration is that of cold fractionation of the oil using a special grade of propane as solvent, the vitamin A being concentrated in one of the fractions so obtained *(54)*. Solvents other than propane have been used for this purpose, and a similar technique has been suggested as a method for separating saturated from unsaturated glycerids in fish oils *(30)*. Vitamin A_2, which probably has a biological value equal to that of vitamin A, occurs in livers and viscera of fresh water fish. Recently a crystalline isomer of vitamin A has been isolated from the high potency liver oils of soupfin shark and halibut, and also from cod livers. This vitamin has been named Neovitamin A, and apparently accounts for 35 percent of the vitamin A in these fish liver oils *(84)*. Of interest is the fact that in young fish vitamin A potency of the oils is low and that it rises very markedly as the age of the fish increases *(83, 96)*. Much remains to be learned regarding the mechanism by which vitamin A is stored in fish livers. Vitamin A is quite unstable in most fish oils, though it appears to be protected in liver oils by natural antioxidants. In this connection it is of interest that very recent work has shown that 0.1 percent of a, β or γ tocopherols, especially when used in combination with 1 percent lecithin, considerably delays oxidative destruction of vitamin A in fish oils *(22)*. The destruction of vitamin A in dry powdered livestock feeds, where it is particularly liable to oxidation, is of economic importance, and methods of stabilizing it are being sought. There is always a danger that fish oils and consequently natural vitamin A will not be as plentiful as they have been, and signs of this are even now in evidence in the rapid depletion of the west coast soupfin shark, dogfish, and possibly pilchard or sardine fisheries. It may be that the time will come when some reliance must be placed in a synthetic source of vitamin A. The task of producing vitamin A in the laboratory is apparently an extremely difficult one. Some success has been achieved, and small amounts of biologically active related compounds have been obtained, though not in commercial quantities *(37, 53, 69)*.

Marine sources of the D vitamins continue to be exploited even with the advent of biologically active substances obtained by irradiation, or similar activation, of related sterols or "provitamins." The reason for this is largely because no provitamin of this type has been prepared by total synthesis, and partly because certain high potency vitamin D oils such as those of halibut and the percomorph fishes can be used directly as clinical sources of these vitamins. Vitamin D occurs largely as vitamin D_2 (viosterol and calciferol) and vitamin D_3 in fish liver oils, though there appear to be at least four other forms of this vitamin present *(38)*. The vitamin occurs partly in the free and partly in the esterified form. Man can utilize both vitamin D_2 and D_3, and fish liver oils are therefore a good source of these vitamins for humans. Vitamin D_2, which can be obtained by irradiation of yeast ergosterol, is apparently not utilized by young chicks, while vitamin D_3 is apparently one hundred percent active in this respect *(14)*. A natural source for a D provitamin for chicks was sought, and it was found that certain marine invertebrates, particularly mussels, yielded a provitamin of this type *(16, 17)*. Commercial use is being made of this discovery. Other marine sources of D provitamins are being sought. Thus one west coast firm is apparently extracting cholesterol, which sells for about $22.00 a pound, from fish meal by a special process which is estimated to cost about $17.00 per ton of treated meal. The yield of cholesterol is said to vary between 2 and

7 pounds per ton of meal. Cholesterol is also present in considerable quantities in certain fish body oils from which it might be prepared. 7-dehydrocholesterol can be obtained from cholesterol, and this in turn irradiated to give vitamin D_3. It has also been found that certain fish liver oils contain a form of vitamin D which is apparently much more active for chickens than vitamin D_3 *(14)*. Some attention is being given to the stabilization of vitamin D in poultry feeds *(32, 36)*.

An interesting development in the oil technology field is that of the use by the Norwegians of certain domestic fish body oils instead of imported olive oil in their canned sardines. The processes of manufacture of such oils apparently involve polymerization, but have remained secret, although the chemical and physical characteristics of the oils have been described *(41, 42, 43, 71, 72)*. Certain fish oils have considerable value for use as fine instrument lubricants. Thus porpoise, jackfish and blackfish jaw oils have long been employed for this purpose *(78)*. Ratfish liver oil *(86)* and Pacific porpoise oils *(95)* should also possess good lubricating properties. Ratfish liver oil mixed with pine oil has been said to have been used for massaging.

Phospholipids, probably lecithin, occur in salmon eggs. They can be obtained from the dried powdered material by first extracting with pentane to remove oil and pigments, and subsequently with a polar solvent such as methanol. Sockeye salmon roe yielded 12.5 percent oil and 6.2 percent phospholipids *(35)*.

MISCELLANEOUS

Numerous miscellaneous marine products have been described, and some of these may have commercial possibilities. Thus bile acids are found in fish, and some idea of the cost of producing these from salmon bile has been given *(26)*. Chitin, a polymerized acetyl glucosamine, is present in 16 to 22 percent concentration in shells of crustacea such as crabs, lobsters and shrimps. Glucosamine can be prepared from chitin by hydrolysis. It has also been suggested that chitin might form a useful alternative to cellulose for making adhesives, varnishes, wrapping materials, sausage casings etc. *(3)*. A number of sterols occur in sponges *(10, 101)* and in marine invertebrates *(9)*. Cholesterol has been found in the sea urchin, and sea cucumbers contain 7-dehydrocholesterol. Different corals have been found to contain cetyl palmitate *(58)*, cholesterol, hydrocarbons etc., and it is thought that coral reefs may play some role in petroleum formation *(8)*.

A rather profitable side line which has been developed on the west coast is that of production of sport fishing baits. Most of the methods used are kept secret, though publications concerning the preservation of salmon eggs *(23, 44)* and small whole fish *(97)* for bait are available. The preservative agents commonly employed in these baits include heat, formaldehyde, alum, borate, benzoates and salt.

Mention must be made before closing of the importance of seaweeds as sources of fishery by-products. It has been known for a long time that seaweeds are good sources of potash and iodine, and commercial use of this knowledge has been made. However, the most interesting products are the polysaccharide-like substances agar, alginic acid and the gelose (carragenin) of Irish moss. Several reviews concerning production methods and properties of these phyco-colloids are available *(67, 87, 88, 90, 98, 99, 104)*. Agar has abundant uses which include microbiology, pharmacy, medicine, dentistry, photography and food manufacture. It occurs in red seaweeds such as those known as *Gelidium* and *Gracilaria*. Alginic acid, which occurs in brown seaweeds such as kelp, and the carragenin of Irish moss, have valuable properties as stabilizers for ice cream, chocolate milk etc A method for determining the stabilizing power of Irish moss extracts has been published recently

(81). Another development in the seaweed field is that of the production of rayon-like fibers from certain metallic alginates *(94)*. It is quite possible that other types of artificial fibers could be made from fish since filaments have been produced from collagen *(40)* and cat-gut like fibers from blood proteins *(25)*.

This review has necessarily been very brief, and it is probably inevitable that much interesting information has been omitted. Undoubtedly several large commercial concerns are carrying on research on fishery by-products, but the results of their work are not often made public. In closing I can only say that I hope that this talk has been of value to you, and that it may stimulate interest and investigation in this subject.

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REFRIGERATOR CAR EXPERIMENTS. VI
A MECHANICALLY REFRIGERATED CAR

O. C. YOUNG, A. W. LANTZ AND D. H. TAYLOR

REFRIGERATOR CAR EXPERIMENTS. VI
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The encouraging results that have attended the work with trucks and trailers in the United States of America led to a study of so called "Parcel Units" (self-contained refrigerator units) employed there. It appeared that these compact units were evolved after many failures with "built-in" units where fatigue in metallic connections was induced from continued vibrations and mechanical shock accompanying ordinary road traffic. Upon reflection it will be realized that railway operation may be even more severe than truck operation because of the lack of rubber tires, therefore it seemed wise to take the lead indicated by the truck experience.

Similarly in connection with the body of the railway car, since considerable expense would be involved in fitting mechanical units into this, it seemed advisable to start with a skeleton car and insulate and remodel it to conform with the latest developments in refrigeration practices. Consequently it was decided to depart somewhat from the orthodox method of cooling by blowing cold air over the bulk of the load, and to equip the car with a "jacket" through which the cooled air could be circulated; and to have the jacket provided with vents leading into the body of the car, so arranged that part of the cooling or heating air (the car was to be adaptable for heating as well as cooling) could be directed through the load.

Toward the latter part of August, 1948, the Canadian National Railways made available to us at Vancouver one of its overhead-cooled type of refrigerator car completely stripped except for the exterior metal sheeting, doors and hatch covers. In this skeleton car a new metal floor was welded, holes were cut in the sides and roof for the two parcel-type units decided upon, and the two compartments in the car for the units were sheathed with metal. Then all the interior seams of the car were sprayed with a waterproofing material (Insul Mastic) to serve as a vapour barrier for the insulation. All the interior members to support the insulation, the modified overhead tanks (described later) and the jacket, were comprised of standard dimensioned lumber, and as a result the thickness of insulation used on the walls is approximately 3⅝ inches with a similar width in the air jacket; the insulation on the floor and ceiling is about 5¾ inches, and that on the ends is rather indefinite because of the corrugations in the metal covering, but would vary between 3⅝ to over 7 inches.

In order that information on the performance of some of the light-weight thermal insulators now being produced might result from these experiments, a number of these different types were used in the car. These included expanded ebonite (Rubatex) and three grades of Fibreglas ranging in densities from 1½ lb. to 4¼ lb. per cu. ft. These insulators were employed in different parts of the car wherever their physical properties lent themselves to easy installation or specific service. For example, the expanded ebonite has structural strength, therefore it was used in certain parts of the floor to carry the weight of the load likely to be carried. At the completion of the experiments with this car, when it is felt that no more significant data can be obtained, the interior can be dismantled and the insulation examined for effects of the treatment to which it was subjected.

Also, in order that the experimental car may have as great a flexibility and wide application as possible, it was fitted with shallow (6-inch deep) aluminium overhead tanks about which the return air to the fans may circulate in its heating or cooling cycle. Auxiliary cooling media such as ordinary ice or dry-ice may be used in these tanks to assist the mechanical units in supplying the required additional refrigeration capacity to, say, cool quickly a carload of fresh fruits or vegetables with the field heat present. Such a load will impose the greatest demand that may be placed on any car, and without any help would require over 24 hours for the units to fulfil. If, by the use of the modified tanks containing 2 tons of ordinary ice, this ice can be melted in about 10 hours through the action of the forced air in circulation about the tanks, then the precooling of the "warm" carload should be accomplished by the combined cooling systems during that 10 hours.

The success of any mechanical system for cooling cars of course depends upon the machines themselves. In these experiments the important features, apart from actual capacities, are reliability and long service under unattended operation. It was realized that the perfect unit for railway cars has not been evolved yet, so the best that could be done was to make a choice from the units giving good results in trucks and trailers, and modify them to fit into our scheme. Having very few past experiences to fall back upon, naturally some unforeseeable difficulties and weaknesses will come to light as the investigation progresses, and these will entail further modifications and adjustments. For these reasons alone it was important that the units give not only reasonable promise of meeting our requirements, but that the company supplying them have sufficient confidence in its equipment and a degree of pioneering spirit to carry it beyond the consideration of immediate sales and profits. Unfortunately no complete units of the type required were being manufactured in Canada; therefore it was necessary to purchase the equipment in the United States of America. Our considered choice was a unit called the "Thermo King," partly manufactured and completely assembled by the U. S. Thermo Control Company of Minneapolis.

As shown in figure 1, two such units are included in these investigations. Each unit is made up of a 4-cylinder gasoline engine driving a 6-cylinder Freon-12 compressor. Each engine can deliver a maximum of 26¼ H.P., but this is three or four times more than the demand of the compressor, so that the engine will never "labour" and thus fail because of an overload. The evaporator and air cooled condenser are so balanced that their functions can readily be reversed to apply the "heat pump" principle. Therefore the units may be used for heating as well as cooling. Eventually the units will be equipped with the necessary automatic devices
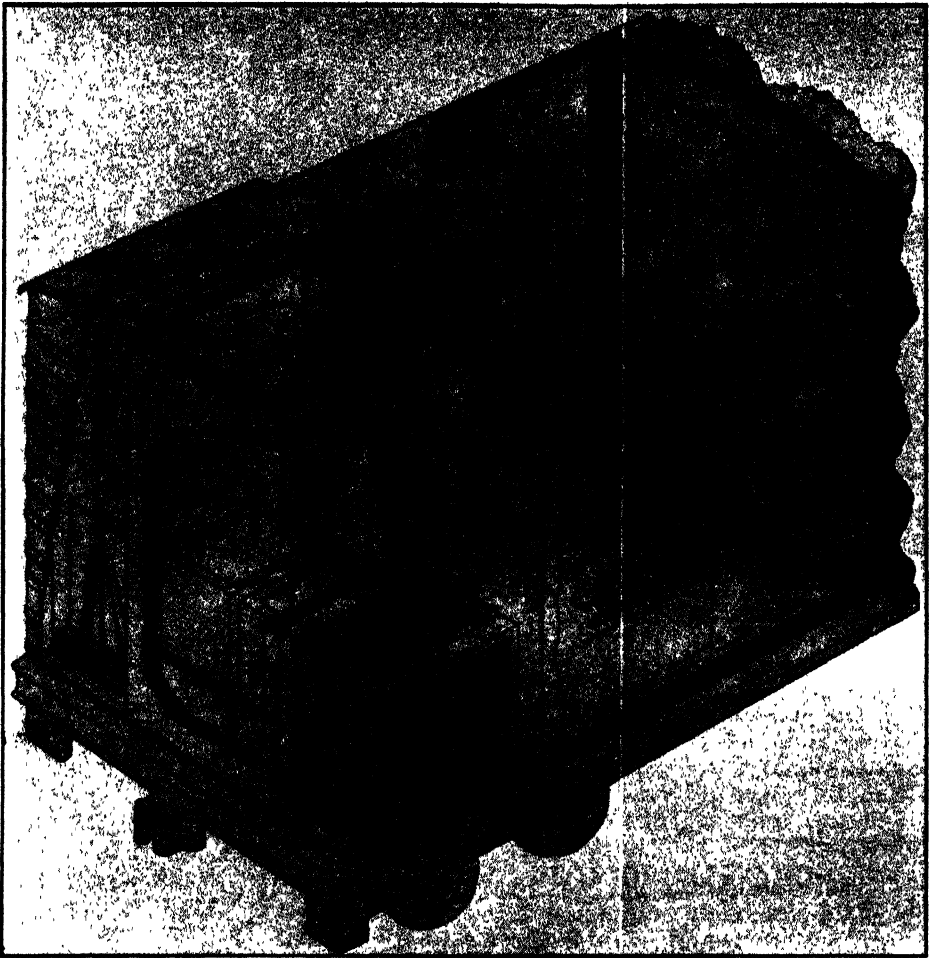
Figure 1.

for self control at any temperature within the range of the thermostats (temperature control devices) under any weather conditions encountered in Canada; but for the earlier experiments the switch-over from "cooling" to "heating" will be done manually. The units are equipped with the ordinary indicating devices found on the modern automobile, but in addition have "hour meters" to show the running time of each unit, so that check-ups and reconditioning can be performed at the proper times.

Reconditioning and overhauling will not be done on the unit while it is in the car; instead, to facilitate a quick change of units if essential, a special clamping device is employed shown in operation in figure 2. By merely disconnecting the fuel and electrical lines the entire unit may be lifted from its base and a reconditioned unit put in its place. This operation need not take more than a few minutes. Furthermore, the component parts of the assembly are joined together with special unions, where necessary, so that a compressor, for example, can be quickly replaced without loss of refrigerant. The above are just a few of the features included to make the experiments as fruitful as possible.

A DYNAMOMETER FOR DETERMINING DEPTH
OF FREEZING IN FOODS[1]

By H. Tessier[2]

Abstract

A dynamometer, designed to determine depth of freezing in frozen foods, such as meat, poultry, and eggs, measures the force required to drive a pointed rod through a sample of the product.

Introduction

The depth of the frozen portion of some food products is frequently a matter of controversy that is often settled by breaking open some units of the frozen food with an ax, thus destroying those units. Often, the depth of freezing is determined by driving a nail into the product. Lack of resistance to the nail indicates an unfrozen portion. This paper describes a dynamometer that will give a semiquantitative estimate of the depth of freezing in foods.

Description

The dynamometer (Fig. 1) consists of a brass cylinder (A) at the lower end of which a chuck (B) holds a removable pointed rod (C). The upper part of the cylinder is threaded to receive a screw cap (D) which clamps down the ringhead (E) of a bellows (F) against a rubber seal (G). A steel rod (H) screwed into the lower part of the bellows (I) guides a metal ram (J). The ram weighs 1 lb. and slides freely over the guide. When this ram is brought down with muscular force against an anvil (K), the oil that fills the space between the bellows and the cylinder permits the pressure exerted by the blow to be indicated on a pressure gauge (L) connected to the cylinder through a bushing (M). To prevent damage to the bellows, a stop (N), soldered to a screw cap (D) limits the upward movement of the bellows when the ram is pulled up against a nut (O) or when the point is pulled out of the sample. For convenience when the instrument is not in use, the guide rod can be unscrewed from the anvil and the pointed rod can also be removed from the chuck.

Procedure

The pointed rod is held against the frozen sample, the ram is gripped firmly with one hand and brought down with force against the anvil until the point penetrates the sample. The pressure at each stroke is noted on the gauge. In a completely frozen sample the pressure required to drive the point into the center of the sample is approximately the same for every stroke, but the

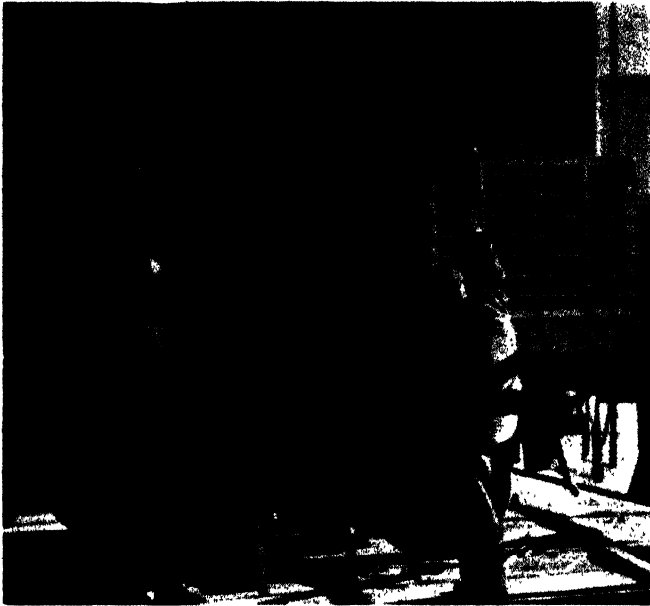# REFRIGERATOR CAR EXPERIMENTS. VI
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Figure 2.

The employment of the particular units decided upon of course reduces the loadable space in the car by about 11% using the 40-foot overhead-cooled car as a comparison. The experimental car has a loadable volume of slightly over 1970 cu. ft. using a height of 7 ft. in the car. The design of the car could be altered to increase this interior height, however, if it is so desired.

The experimental car was ready for some of the preliminary tests by mid-October. Only one unit was installed at that time, since the action of shock and switching in local yards was to be observed first. Any weaknesses that might be revealed in shock tests could then be corrected in the second unit. The U. S. Thermo Control Company had its own engineer, Mr. C. K. Dunn, who installed the unit, make observations in all preliminary tests so that any modifications suggesting themselves as a result of the tests could be carried out in its factory.

Only two tests giving significant results have been conducted so far. The first of these was to test the security of the clamping devices for the unit. For this purpose the Canadian National Railways supplied a Savage Impact Register, by means of which a chart showing the magnitude of the shocks imparted was obtained. Mechanical shocks were imparted to the experimental car by yard tests, both by "hitting" it with an engine moving at different speeds, or letting it hit a stationary string of cars while moving at different speeds. The Impact Register indicated that impacts up to the wrecking point (10-11 miles per hour) were imparted to the car, with little evident effects on the equipment or its operation.

The second significant test was a short road test in which a shipment of 24,540 lb. of frozen foods was made, accompanied by Mr. Dunn and one of us (D.H.T.), from New Westminster, B.C. to Edmonton, Alberta. The outside temperatures were not conducive to the obtaining of convincing

cooling results, so only the one unit was operated during the trip which took slightly under three days. The main object of this road test, therefore, was not merely to induce sub-zero temperatures in the car, but rather to determine whether the equipment would perform satisfactorily under ordinary railway operating conditions.

The observations made on this trip may be briefly summarized as follows: The outside temperature at time of loading was approximately 43°F. In the car the mean temperature of the bottom air was +2.5°F.; that of the top air was +18.2°F.; and the temperature of the product (centre of load near top) was +3.2°F. When the unit was shut down nearly three days later, the outside air was 22°F. (the mean for the trip being 32.1°F.). Inside the car the mean bottom air was −18.5°F.; the mean top air was −12.2°F., and the product (top centre) was −6.3°F. Approximately 53 Imperial gallons of ordinary grade gasoline was used for the entire test including the precooling period of something like 2 hours. Analysis of the results indicate that the equipment has the capacity expected and that it will operate satisfactorily under railway service. However, the temperature records showed that the air distribution is not uniform when only one unit is operating; so that some adjustments will be necessary to direct the air more evenly in future experiments.

Further road tests for both heating and cooling are being planned to take place when the weather conditions are suitable to give convincing results. It is hoped that the heating tests will take place early in 1949, and the cooling tests in the summer of 1949.

Acknowledgment

Pacific Fisheries Experimental Station

O. C. Young
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By H. Tessier[2]

Abstract

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The dynamometer (Fig. 1) consists of a brass cylinder (A) at the lower end of which a chuck (B) holds a removable pointed rod (C). The upper part of the cylinder is threaded to receive a screw cap (D) which clamps down the ringhead (E) of a bellows (F) against a rubber seal (G). A steel rod (H) screwed into the lower part of the bellows (I) guides a metal ram (J). The ram weighs 1 lb. and slides freely over the guide. When this ram is brought down with muscular force against an anvil (K), the oil that fills the space between the bellows and the cylinder permits the pressure exerted by the blow to be indicated on a pressure gauge (L) connected to the cylinder through a bushing (M). To prevent damage to the bellows, a stop (N), soldered to a screw cap (D) limits the upward movement of the bellows when the ram is pulled up against a nut (O) or when the point is pulled out of the sample. For convenience when the instrument is not in use, the guide rod can be unscrewed from the anvil and the pointed rod can also be removed from the chuck.

Procedure

The pointed rod is held against the frozen sample, the ram is gripped firmly with one hand and brought down with force against the anvil until the point penetrates the sample. The pressure at each stroke is noted on the gauge. In a completely frozen sample the pressure required to drive the point into the center of the sample is approximately the same for every stroke, but the

pressure required and the depth of penetration at each stroke varies with the temperature and the type of goods. In a partly frozen sample a drop in pressure is noted when the point penetrates into the unfrozen portion. For
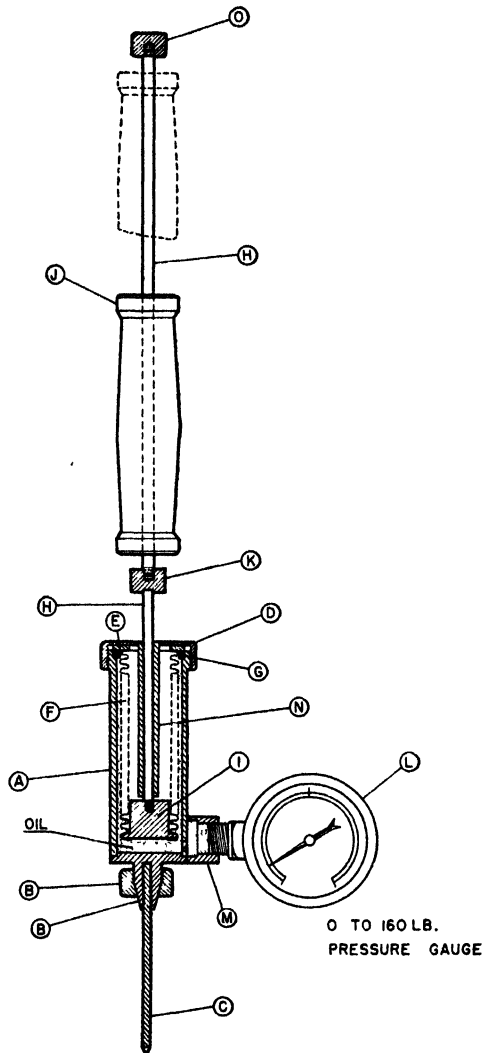

FIG. 1. *Dynamometer for frozen foods.*

example, poultry at 30° F. offers no resistance to the dynamometer, poultry at 20° F. offers about a 50 lb. resistance, and at 0° F. about 160 lb. resistance.

Preliminary trials have indicated that this instrument can be used for inspection work on frozen poultry as a rapid method of detecting unfrozen spots in the center of a carcass. It should be equally useful for determining depth of freezing in other types of frozen flesh, in frozen egg, in fruits frozen in syrup, and in other frozen products.

CANADIAN ERUCIC ACID OILS

III. SHORTENINGS FROM RAPE AND MUSTARD SEED OILS

By H. J. Lips, N. H. Grace, and Suzanne Jegard

CANADIAN ERUCIC ACID OILS[1]

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By H. J. Lips,[2] N. H. Grace,[2] and Suzanne Jegard[3]

Abstract

Processed rape and mustard seed oils were hydrogenated at 50 lb. pressure and 284° F., using a commercial nickel formate catalyst, and were deodorized for one hour at 464° F. The stability of the hardened oils compared favorably with that of a standard commercial vegetable shortening. Taste panel tests indicated that both the rape and mustard shortenings were as satisfactory as the commercial reference material for the preparation of pastry and doughnuts. Special study of the rape product showed that it could be plasticized, and it gave good results in baking volume experiments.

Introduction

In the nonselective hydrogenation of vegetable oils commonly employed in the manufacture of shortening, some (combined) stearic acid is formed by complete saturation of oleic acid, and some elaidic acid by geometrical transformation of oleic acid (2, p. 565). The comparable reactions in erucic acid oils would lead to the formation of behenic and brassidic acids from erucic acid. In view of the widely different melting points and structures of the possible fatty acid products, it might be expected that shortenings from erucic acid oils could differ markedly from those in ordinary use. Moreover, Canadian shortening manufacturers have suggested verbally that hardened erucic acid oils may be inferior in baking volume properties. On the other hand, Canadian rape and mustard seed oils were shown to possess potential value as salad and cooking materials (6), and hardened rape seed oil was eaten during the war in Germany (4), so it might be expected that shortenings from rape and mustard seed oils would be acceptable food products. Estimation of possible consumer reaction to shortenings prepared from these oils by a standard method was therefore undertaken, with special consideration to the effect of rape shortening (the more important product) upon baking volume.

Materials and Methods

The processing of the crude rape and mustard seed oils used in this work has been described (3, 6). In brief, they were refined with 10° Bé. alkali and bleached under nitrogen with 2% Superfiltrol for 20 min. at 212° F.

The oils were then hydrogenated in a pilot-scale, stainless steel hydrogenator of 10 lb. capacity, at 50 lb. pressure and 284° F., with purging at 15 min. intervals. The catalyst was commercial nickel formate (25% nickel) at

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[3] *Biochemist, Food Investigations.*

1% concentration. The course of hardening was followed by withdrawing samples and determining the refractive index. When the desired hardness was reached, the material was removed from the hydrogenator, 1% diatomaceous earth was added, and the mixture was filtered in a porcelain Büchner funnel. The shortening was then steam deodorized under vacuum in an all-glass apparatus for one hour at 464° F. Each test shortening was a composite of two or three individual preparations. A standard commercial vegetable shortening of high quality was used as reference material in all tests, and comparisons were made both with fresh shortenings and shortenings aged for 10 days at 100° F.

Color, fluorescence, kinematic viscosity, peroxide oxygen, free fatty acid, and smoke point were measured at different stages of use of these materials, as previously described (3, 6). Iodine value was determined by a modification of Wij's method (5) and melting point by the capillary tube method (1).

Pastry and doughnuts were prepared with the shortenings according to the recipes given in Table I. Pastry was baked 10 min. at 435° F. and served at

TABLE I

RECIPES FOR DOUGHNUTS AND PASTRY, GIVEN IN GRAMS

Constituent	Doughnuts	Pastry
Eggs	96	—
Flour	500	112
Sugar	200	—
Shortening	25	66
Milk	224	—
Water	– –	30
Baking powder	15	—
Cinnamon	$\frac{1}{2}$	...
Nutmeg	$\frac{1}{2}$	—
Salt	4	2

room temperature; doughnuts were fried in the shortenings at 355° to 365° F. for three minutes and served warm. Pastry and doughnuts were scored for taste and odor by a 24-member panel as previously described (6); excess or deficiency of the property was rated on an integral scale of + 5 to − 5.

After some preliminary trials, the following biscuit volume method (Method A) was developed to determine the effect of shortenings on baking volume: 100 gm. of flour, 3.5 gm. of baking powder, and 1.3 gm. of salt were mixed for one minute in a jacketed dough mixer held at 100° F. Fat (17 gm.) warmed to a temperature of 140° F. was added to the dry mixture and the mixing process continued for another minute, then 50 ml. of water was added and mixed in for one minute more. The total mixing time was three minutes. A 100 gm. sample of this batter was weighed into a small baking tin and baked for 30 min. at 475° F. The volume of the baked biscuit was measured

by a rapeseed displacement method (7). In variations of this procedure, 60 ml. of water was used instead of 50 ml. (Method *B*); and unmelted rather than melted fat was used (Method *C*).

Results

Shortening Measurements

Tables II and III present physical and chemical measurements for the commercial, mustard, and rape shortenings. The commercial shortening had a lower iodine value and melting point (Table II) than the rape and mustard

TABLE II

CHARACTERISTICS OF SHORTENINGS USED FOR TASTE PANEL TESTS

Shortening	Melting point, ° C.	Iodine value
Commercial	38.0	57.9
Mustard	42.9	67.6
Rape	45.4	58.3

shortenings of about the same hardness chosen for comparative test. The relative transmissions at 440 and 660 mμ of the rape and mustard products were similar, and slightly less than those of the commercial material (Table III). Indications were (Table III, *C*) that the color was not sufficiently intense to interfere with the measurement of fluorescence. Fluorescence in ultraviolet light (375 mμ) increased in the order: rape, commercial, mustard; and viscosity in the order: commercial, mustard, rape. Peroxide and free fatty acid values were uniformly low. The commercial shortening had the highest smoke point, with rape shortening next, and mustard shortening lowest but still fairly satisfactory.

These characteristics were altered in varying degree by aging the shortenings or by using them for the deep fat frying of doughnuts (Table III): transmission was not changed by aging but decreased markedly with frying; fluorescence increased with frying; viscosity was practically constant;

TABLE III

CHEMICAL AND PHYSICAL MEASUREMENTS ON SHORTENINGS
AT VARIOUS STAGES OF USE

Shortening	Condition of shortening			
	Fresh	Aged	Fresh, fried	Aged, fried
	Measurement			
	A. Transmission, per cent at 440 mμ and 60° C., relative to mineral oil (Stanolax)			
Commercial	68	68	48	56
Mustard	58	55	38	36
Rape	61	61	48	35

TABLE III—*Concluded*

CHEMICAL AND PHYSICAL MEASUREMENTS ON SHORTENINGS
AT VARIOUS STAGES OF USE—*Concluded*

Shortening	Condition of shortening			
	Fresh	Aged	Fresh, fried	Aged, fried
	Measurement			
	B. *As in A, at 660 mμ*			
Commercial	98	97	95	97
Mustard	92	92	96	96
Rape	90	91	95	94
	C. *Transmission, per cent at 440 mμ of 1 gm. of shortening in 100 ml. xylol, relative to xylol*			
Commercial	99	100	99	100
Mustard	99	99	99	99
Rape	100	99	99	98
	D. *Fluorescence, Coleman photofluorometer units, 1 gm. of oil in 100 ml. xylol, corrected for fluorescence of xylol*			
Commercial	16	13	18	18
Mustard	32	33	38	38
Rape	12	10	19	17
	E. *Viscosity, centistokes at 130° F.*			
Commercial	27 2	27 6	27 2	27 5
Mustard	30.6	30 4	30 2	30 2
Rape	33 3	33 8	34 0	33 8
	F. *Peroxide value, ml. of 0 002 N thiosulphate per gm.*			
Commercial	0 0	0 0	2.8	4 9
Mustard	0 0	0 0	4.3	3 9
Rape	0 0	0 0	3 8	3 7
	G. *Free fatty acid content, as % oleic acid*			
Commercial	—	—	0 1	0 1
Mustard	—	—	0.2	0 2
Rape	—	---	0 2	0 2
	H. *Smoke point, ° F.*			
Commercial	430	427	398	399
Mustard	387	385	385	372
Rape	414	410	388	382

peroxides were detectable only in the fried shortenings; smoke points were · lowered slightly by aging, and to a greater extent by frying.

Taste Panel Tests

Taste panel ratings for all pastry and doughnut products (Table IV) showed no difference between doughnuts prepared from any of the types of shortening tested but, on the average, odor and flavor of pastry made with the experimental fats were rated as slightly preferable to those of pastry made with similar amounts of the commercial shortening.

TABLE IV

AVERAGE PANEL SCORES FOR ODOR AND FLAVOR OF PASTRY AND DOUGHNUTS

Shortenings	Pastry		Doughnuts	
	Odor	Flavor	Odor	Flavor
Fresh:				
Commercial	+ 0 5	+ 0 4	+ 0.1	+ 0 1
Mustard	+ 0.1	+ 0 1	+ 0 2	+ 0.1
Rape	+ 0.2	+ 0 2	+ 0.1	+ 0 2
Aged:				
Commercial	+ 0 5	+ 0 7	+ 0.2	+ 0 2
Mustard	+ 0.1	+ 0 2	+ 0.2	+ 0 2
Rape	+ 0 1	+ 0 2	+ 0.2	+ 0 2
Necessary difference (5% level of statistical significance)	± 0.4	± 0.4	± 0 3	± 0 4

Baking Volume

Different samples of hydrogenated rapeseed oil, with iodine values from 40 to 100, showed no significant volume difference by baking methods *A* or *B* in two series of hydrogenated oils (Table V).   The additional water added in Method *B* caused a general increase in baking volume that was significant for most of the shortenings tested.   However, when unmelted fat was used (Method *C*), the rapeseed biscuits were significantly smaller than the reference biscuits if the hydrogenated oil had an iodine number less than about 85. Biscuits made from the commercial product had the same baking volume regardless of the state of the fat.   These results indicated that when the unmelted rapeseed shortening was used some physical factor interfered with fat distribution in the mixture and resulted in an inferior baking volume.

The hydrogenated rapeseed oil was then plasticized to make its consistency approximate more closely that of the standard commercial shortening.   The steps in the plasticizing process were: (*a*) preliminary heating of the fat to 212° F., (*b*) beating of the melted fat in a Mixmaster with rapid cooling at −40° F. until the mass solidified, and (*c*) tempering the plasticized product at 80° F. for three days.   Two samples of plasticized fat were prepared from

TABLE V

COMPARATIVE BISCUIT VOLUMES FOR HYDROGENATED RAPESEED OILS
AND A COMMERCIAL VEGETABLE SHORTENING

(Averages of three determinations)

Description of samples			Biscuit volume, ml.		
Shortening	Iodine No.	M.p., ° C.	Method *A* (50 ml. water)	Method *B* (60 ml. water)	Method *C* (un-melted fat)
Standard (commercial vegetable shortening)	57.9	38.0	195	210	195
Hardened rapeseed oils, samples taken at intervals during hydrogenation:					
Hydrogenation series I	103.2	< 15 0	204	208	-—
	90.0	18.0	199	212	- -
	65 9	42 0	200	214	—
	49 2	49 8	196	—	- -
	44.5	52 0	199	210	—
Hydrogenation series II	100 5	< 15 0	198	—	194
	87.8	23 3	202	—	186
	63 8	42 8	197	—	183
	55.0	47 6	197	—	184
Necessary difference (5% level of statistical significance)			10		

a rapeseed shortening of melting point 46° C., which was higher than the melting point of the commercial shortening (38° C.). The plasticized products were found to have good mixing properties in the solid form. Biscuits were baked with the fat in an unmelted state. The resulting volumes were:

Unplasticized — 184 ml.

Plasticized sample No. 1 — 191 ml.

Plasticized sample No. 2 — 196 ml. (a significantly higher figure than for the same material unplasticized)

Commercial — 195 ml.

These figures showed that the hydrogenated rapeseed oil could be plasticized to give as good a biscuit volume as the standard commercial vegetable shortening.

Conclusions

Hardened erucic acid oils prepared by a standard method did not differ markedly from ordinary shortening in the properties studied, in spite of inherent compositional differences. No special hardening techniques were necessary to prepare shortenings acceptable as food products from processed

Canadian rape and mustard seed oils.   These shortenings were stable materials of fair color and smoke point, and were amenable to plasticization and use in baking.

Acknowledgments

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DRIED WHOLE EGG POWDER

XXVIII. REPRODUCIBILITY AND INTERRELATION OF METHODS OF ASSESSING QUALITY[1]

BY JESSE A. PEARCE[2] AND M. W. THISTLE[2]

Abstract

The relation between palatability and fluorescence value, previously established for 33 samples of plain egg powder, was substantiated by comparisons for 118 samples. Fluorescence measurements were more readily reproduced among various laboratories than measurements of potassium chloride value. Batter density measurements were found to be a satisfactory measure of the baking quality of sugar–egg powder and were more convenient than the baking of test cakes. Particle size of spray-dried sugar–egg powder was also related to baking quality; powder falling between 50 and 200 mesh (U.S. Bureau of Standards) yielded the lightest sponge goods.

Introduction

During the course of work reported in earlier papers in this series, several tests of quality in plain dried egg powder were described and some of these have been written into standards governing quality (1, 2, 5). The reproducibility of these tests became the subject of collaborative studies. On sugar–egg powder, measurements of baking volume were inconvenient for control testing and simpler methods were sought. Some of these were also assessed in a collaborative study.

The present paper, describing the results of this work, is divided into two main parts. The first deals with studies on plain egg powder and describes further assessment of the fluorescence–palatability relation (7), and collaborative measurements of fluorescence (8) and potassium chloride values (11). The second deals with studies on sugar–egg powder; it describes the relations between a number of measurements and cake volume (6), and a collaborative study of foam density, batter density (5), and cake volume (5) as methods of assessing baking quality.

Plain Egg Powder

The Palatability–Fluorescence Relation

In the first report (7) on the relation between palatability scores and fluorescence value a correlation coefficient of $-.90$** was obtained for a 14-man panel testing 33 samples of dried egg. The equation relating these characteristics was

$$y = 2.32 - 0.12\,x,$$

where y is the logarithm of the photofluorometer reading and x is the palatability rating on a scale ranging from 10 for excellent to 0 for inedible samples.

** *Exceeds the 1% level of statistical significance.*

In the present study, eating-quality assessments by a 12-man panel on 118 samples over a wide range of quality were found to be related to fluorescence values (8) by the equation

$$y = 2.28 - 0.11\,x$$

with a correlation coefficient of $-.84^{**}$. Neither of the parameters of this equation differed significantly from those previously established, and there was no significant difference between the two correlation coefficients.

Collaborative Study of Fluorescence and Potassium Chloride Values

To provide material for this study, a sample of good egg powder was thoroughly mixed, divided into four lots, treated as shown in Table I, canned in 100-gm. samples in No. 1 tins, and held at $-40°$ F. Samples from each

TABLE I

FLUORESCENCE AND POTASSIUM CHLORIDE VALUES ON EGG POWDER OF FOUR QUALITY LEVELS

(Rounded averages of 24 determinations)

Sample designation	Treatment, days at 110° F.	Fluorescence value	Potassium chloride value
Good	None	22	71
	2	29	68
Poor	5	49	43
	9	68	34

treatment were distributed (by air express where necessary) under randomized code numbers, at monthly intervals for four months, to each of the three co-operating laboratories. Each laboratory determined the fluorescence (5) and potassium chloride values (5) on duplicate samples from each tin.

Since duplicate analyses on 'poor' quality powders were significantly more variable than those for 'good' quality powders, the data had to be divided for separate statistical analyses.

The fluorescence measurements made by Laboratory 2 on poor quality egg powders (Table II) were lower than the measurements made in the other two laboratories but, for good egg powders, there was no significant difference attributable to laboratories. The potassium chloride values determined in Laboratory 1, for both good and poor quality powders, were significantly lower than measurements made in either of the other two laboratories. Laboratory 3 consistently recorded high values for the 'good' sample with an average potassium chloride value of 68 (Table I).

An assessment based upon the differences between laboratories, within laboratories, between replicate samples, and between duplicate analyses is given in Table III. The average difference between any two fluorescence

** *Exceeds the 1% level of statistical significance.*

TABLE II

FLUORESCENCE AND POTASSIUM CHLORIDE DETERMINATIONS AT THREE LABORATORIES FOR
'GOOD' AND 'POOR' SAMPLES OF DRIED EGG POWDER

(Averages of 16 determinations)

Laboratories	Fluorescence		Potassium chloride	
	Good samples	Poor samples	Good samples	Poor samples
1	26.5	58.9	67.7	35.3
2	24.8	56.2	69.4	40.6
3	25.0	59.8	70.4	39.6
Necessary difference, 5% level of significance	—	2.7	1.4	2.3

TABLE III

ESTIMATED TOTAL ERRORS OF DETERMINATIONS OF FLUORESCENCE AND
POTASSIUM CHLORIDE VALUES

(Based upon results from three laboratories)

Estimates of error	Fluorescence		Potassium chloride	
	Good samples	Poor samples	Good samples	Poor samples
Standard deviation of a single observation	1.6	3.0	2.0	3.5
Average difference between two observations selected at random	1.8	3.4	2.3	4.0
'Maximum' difference between two observations selected at random (5% point)	4.7	9.0	6.1	10.5

value determinations on a good egg powder, under the conditions of this experiment, was likely to be less than 2 units, and the 'maximum' difference (5% point) was likely to be less than 5 units. The corresponding figures for the potassium chloride determination were 2.5 and 6.5 units. Of the two methods of assessing egg powder quality, the fluorescence method was better than the potassium chloride method from the standpoint of reproducibility of results, since, in addition to random errors, determinations of the potassium chloride values were in error because of systematic differences between one laboratory and another.

Sugar–Egg Powder

Since sugar–egg powder is intended for use in baked goods, its baking quality must be controlled. But the baking of test cakes is often inconvenient, and a simpler method of measuring baking quality was desired.

Foaming volume measurements provided useful information in some of the initial experimental work (3), but it was observed that this measure was subject to irregularities (4), and it did not appear to be satisfactory for controlling commercial production.

In the present work, the relations between cake volume and a number of other characteristics were examined, with special attention to determinations of particle size. This was followed by a collaborative test of the cake volume methods in current use. Simple density measurements on cake batter had been suggested as a possible test of baking quality, and density measurements were therefore included in this collaborative study.

Relation Between Some Quality Tests and Cake Volume

The powder used in this part of the study was produced in two Canadian plants using cone-type driers during the fall of 1945: 22 samples were taken at various times from the main drying chamber of one plant and nine samples from the main chamber of the other. The samples were produced under a variety of conditions and represented the range of quality likely to be met in commercial operations.

The analytical measurements made on these powders included determinations of moisture content (11), fluorescence value (8), potassium chloride value (11), pH (5), foaming volume (4, 6, 9), foam stability (6), and cake volume (6). In addition, packing density was determined by measuring the volume occupied by 1 lb. of powder when poured into a container and subjected to three free falls of four inches. Finally, particle size determinations were made by sieving a 1 lb. sample on a Ro-tap shaker for one hour and determining the weight of powder in the various mesh ranges used (U.S. Bureau of Standards). Although this does not provide an exact separation of the powder (10), it was believed satisfactory for the present purpose.

Most of these tests were useless from the standpoint of prediction of cake volume because either the correlation coefficients were too low or different equations had to be used for powders from different plants. Foaming volume was again related to baking volume ($r = .73**$). However, sieve analysis of powders as received from the driers provided the best estimate of cake volume ($R = .80**$), a fact that seemed to warrant further work.

Relation Between Particle Size and Cake Volume

In a preliminary study, an attempt was made to assess the effect of various drying conditions on batches of spray-dried powder of uniform particle sizes, to be obtained by using a fixed nozzle aperture, but this failed because these other drying conditions also had an effect on particle diameter.

During the winter of 1946, cakes were baked using 30 samples of sugar–egg powder from the main drying chambers of eight plants and 14 samples from the secondary collectors of three plants. Samples of the same powders were

*** Exceeds the 1% level of statistical significance.*

sieved as previously described. The prediction equation for powders from the main chambers was:

$$y = 229.3 - 0.094x_1 + 0.556x_2 + 0.450x_3 + 0.453x_4,$$

where y is the cake volume in ml. and x_1, x_2, x_3, and x_4 are the percentages of the powder falling between mesh sizes 16 and 50, 50 and 80, 80 and 100, and 100 and 200 respectively. The same equation could be used for powders from the secondary collectors, except that the first term was reduced from 229.3 to 213.3; i.e., on the average, powders from the main chambers gave cakes 16 ml. larger than samples with the same sieve analysis from secondary collectors. However, for both equations, the correlation coefficient was only .75** and the standard error of estimate was ±11.4 ml. This showed that sieve analysis alone was not a satisfactory measure for controlling the baking quality of commercially produced sugar–egg powders.

These equations indicated that attention should be given to adjusting the conditions of spray-drying to produce powders finer than 50 mesh. Optimum cake volume at 80 to 100 mesh was suggested by an earlier study (6). The poor baking quality of the largest particles may be due to the fact that they dry most slowly and deteriorate while they are wet.

Collaborative Study of Tests of Baking Quality

Since the foregoing work yielded no satisfactory substitute for cake volume measurements, it seemed advisable to examine the baking tests in current use, and coincidentally, to reassess the possibility of using foam density and to assess batter density for predicting baking quality.

For this study, 30 samples of spray-dried sugar–egg were drawn from Canadian commercial production during the summer of 1946: 20 samples were obtained from main drying chambers and 10 samples from secondary collectors. Each sample was thoroughly mixed in a mechanical device and aliquots were packed in No. 1 tins and sent to the participating laboratories for triplicate analyses. The procedure described elsewhere (5) for determining batter density and cake volume was used with minor modifications* by most of the laboratories. Laboratory 1 used a simpler procedure (6). Laboratory 5 used both procedures and their results for the simpler procedure are listed as Laboratory 5A. Foam density was determined on the batter before the addition of flour.

For powders from the main drying chambers, differences between samples were nearly all within the limits of experimental error and hence failed to provide any information on the interrelations under study. For secondary collector powders, the correlation coefficients were considerably higher and most of them were statistically significant (Tables IV and V).

* *The details, if desired, can be obtained by requesting a copy of Interim Report No. 46-12-1, Division of Applied Biology, National Research Laboratories, Sussex St., Ottawa.*
** *Exceeds the 1% level of statistical significance.*

TABLE IV

INTERRELATION BETWEEN THE METHODS USED BY FIVE LABORATORIES FOR ASSESSING THE BAKING QUALITY OF 10 SAMPLES (SECONDARY COLLECTOR) OF SUGAR–EGG POWDER

(Simple correlation coefficients between laboratories)

Methods	Lab. No. 2	Lab. No. 3	Lab. No. 4	Lab. No. 5	Lab. No. 5A
CAKE VOLUME					
Lab. No. 1	.81**	.92**	.88**	.82**	.71*
Lab. No. 2		.75*	.60	.59	.52
Lab. No. 3			.78**	.66*	.74*
Lab. No. 4				.89**	.75*
Lab. No. 5					.85**
FOAM DENSITY					
Lab. No. 1	.52	.87**	.75*	.79**	
Lab. No. 2		.51	.50	.63	
Lab. No. 3			.86**	.80**	
Lab. No. 4				.64*	
BATTER DENSITY					
Lab. No. 1	.59	.86**	.67*	.93**	
Lab. No. 2		.55	.61	.70*	
Lab. No. 3			.75*	.92**	
Lab. No. 4				.74*	

* *Attains 5% level of statistical significance.*
** *Attains 1% level of statistical significance.*

TABLE V

INTERRELATION BETWEEN THE METHODS USED FOR ASSESSING THE BAKING QUALITY OF 10 SAMPLES (SECONDARY COLLECTOR) OF SUGAR–EGG POWDER, AT EACH OF FIVE LABORATORIES

(Simple correlation coefficients between methods)

Laboratories	Cake volume × foam density	Foam density × batter density	Cake volume × batter density
1	− .88**	.99**	− .87**
2	− .61	.84**	− .87**
3	− .84**	.99**	− .85**
4	− .66*	.83**	− .79**
5	− .95**	.86**	− .88**
5A	− .79**	.86**	− .87**

* *Attains 5% point of statistical significance.*
** *Attains 1% point of statistical significance.*

Table V shows the interrelation, within laboratories, between the various methods of measuring baking quality. At some laboratories, correlation coefficients between cake volume and foam density were too low for prediction purposes. The relation between foam density and batter density is of little practical importance. However, the highly significant correlation coefficients for the relation between cake volume and batter density indicated that a batter density test might be useful for measuring baking quality. Calculations

showed that sugar–egg powder giving a batter with density greater than 0.43 by any of the procedures used was unsuitable for use in the best class of sponge goods. Therefore, during the past two years, a batter density test (5) has been used to control the baking quality of sugar–egg powder exported from Canada.

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Ammonium Nitrate Brines Lower Refrigerator Car Temperatures

Final Road Tests Indicate an Appreciable Temperature Drop but Economic Feasibility is Questioned

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LABORATORY experiments[1] have shown that the addition of 15 lb ammonium nitrate to the conventional mixture of 100 lb ice plus 30 lb sodium chloride lowered the bunker temperature approximately 10 deg. In subsequent road tests[2] the addition of similar amount of ammonium nitrate reduced the average

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Previous papers, published in March and July, 1948, issues of REFRIGERATING ENGINEERING, described laboratory investigations and two practical operating trials on ammonium nitrate—sodium chloride—ice mixtures to produce lower temperatures in the overhead brine bunker type of refrigerator car. The present paper describes a third road trial in which average temperatures were lower in the test car than in the car employing only sodium chloride and ice by the following amounts: 8.2 deg F in the bunkers, 5 deg F in the car air, 3 deg F in the product during transit, and 4.4 deg F in the product at destination.

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bunker temperature 7 deg F. Individual temperature readings, however, indicated that the new cooling mixture was affected more than the conventional sodium chloride—ice mixture by the size of ice and the frequency of icing. A further road test was therefore undertaken to produce more uniform bunker conditions and thereby to ascertain the minimum temperature attainable with the new mixtures.

Test Conditions

This road trial was conducted during June and July, 1948, over the Canadian National Railways from Lunenburg, N. S., to western Canada (previous trials were from west to east), with boxed frozen fish (fillets)

as the test commodity. The boxes used on the present trial were much smaller and therefore constituted a more densely packed loading with few voids for air circulation.

Most of the test conditions and temperature-measuring arrangements were similar to those used on previous trials[2] except that no attempt was made to measure product—air temperatures; instead, 18 thermocouples per car were frozen in separate boxes of produce to give a better estimate of the true product temperature.

The Control Car, (T.C.) was iced with 30 lb sodium chloride per 100 lb ice and the Test Car (T.A.) employed 15 lb ammonium nitrate plus 30 lb sodium chloride per 100 lb ice.

However, experience had shown that the high solubility and rapid rate of solution of ammonium nitrate caused most of this salt to dissolve within a few hours after icing. This meant that low temperatures were observed initially, followed by a rise in temperature as the solution was diluted from additional ice meltage. This together with losses through the brine overflow tended to decrease the ammonium nitrate concentration in the bunkers. Laboratory tests[1] have shown that temperatures of —15 F are attained when 15 lb of ammonium nitrate per 100 lb ice are added, but at this temperature the concentration in solution was about 20%. In this road test the concentration of ammonium nitrate *in solution* was estimated and extra charges were added when the concentration fell below 20%.

In general the cars were iced once every 18 hr but the actual intervals varied from 9 to 35 hr. Very coarse ice was received only twice throughout the trial.

Results and Discussion

Figure 1 shows average temperatures within and between days. The top air temperature was consistently cooler than the bottom air temperature, a result attributable to the tight packing of the load.

A summary of the average temperatures and ice and salt consumption of the two cars appears in Table 1. The addition of ammonium nitrate during the comparable period lowered the bunker temperature 8.2 deg F as compared with the control car. This value is still short of the 10 deg F lowering obtained in the laboratory investigations. Improved schedules for adding ammonium nitrate might produce still lower bunker temperatures but it seems probable that the present figures are typical of what would be obtained in operating practice.

True temperature in the car was probably lower than the observed temperatures since the thermocouples were concentrated in the southeast quarter of the loadings, the section exposed to the sun. The 8.2 deg reduction in bunker temperature reduced the internal air temperatures almost 5 deg. The product temperature, essentially comparable for the two cars at loading, averaged 3 deg lower in the car receiving ammonium nitrate over the period of transit and was actually 4.4 deg lower at destination. These figures also underestimate the performance of the ammonium nitrate car since the product temperature in the control car increased 2 deg in transit whereas the product temperature was reduced over 2 deg in the test car. It is

obvious that the cooling of a product represents a greater refrigerating load than the maintenance of a uniform product temperature.

The additional reduction in temperature cannot be obtained without additional expenditure. The test car consumed 2000 lb ice, 600 lb sodium chloride, and 3260

Table 1. Summary of Average Temperatures, Ice and Salt Consumption, and Time in Transit

Item	Car T.C.*	Lunenburg to Winnipeg Car T.A.**	Difference	Lunenburg to Edmonton Car T.A.**
Outside air (shade)	64.8 F	64.8 F	—	65.4 F
Bunkers	—3.6 F	—11.8 F	8.2 deg F	—11.5 F
Car air (top)	8.7 F	3.9 F	4.8 deg F	4.7 F
Car air (bottom)	11.5 F	6.6 F	4.9 deg F	7.1 F
Product—loading	8.5 F	8.4 F	0.1 deg F	8.4 F
Product—transit	10.1 F	7.1 F	3.0 deg F	7.4 F
Product—destination	10.7 F	6.3 F	4.4 deg F	7.9 F
Ice consumed	16,000 lb	18,000 lb	2,000 lb	24,600 lb
Sodium chloride consumed	4,800 lb	5,400 lb	600 lb	7,380 lb
Ammonium nitrate consumed	—	3,260 lb	3,260 lb	3,810 lb
Time in transit	131 hr	131 hr	—	205.5 hr

* Car T.C.—30 lb sodium chloride per 100 lb ice.
** Car T.A.—15 lb ammonium nitrate plus 30 lb sodium chloride per 100 lb ice.

lb ammonium nitrate more than the control car over the comparable period. Whether the contribution to product quality obtained by the lower temperature will justify the actual cost remains a problem for the future. It is obvious, however, that loading facilities which will enable the product to be loaded on the car at the desired transit temperature are essential if the full effect of low temperature refrigeration is to be realized.

Acknowledgements

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Discussion

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We have all heard much theory in past years about eutectic ices and brines for transportation of perishables, but this is the first time someone has tied the eutectic principle down to some sort of practical railroad operation. Dr. Cook's research in both lab and field has been most thorough.

The chief facts which Dr. Cook has demonstrated seem to be that the practical eutectic points in car bunkers are: for NaCl and water ice, —5 F (30 to 100 lb ratio); for ammonium nitrate and water ice, about 3 F (56 to
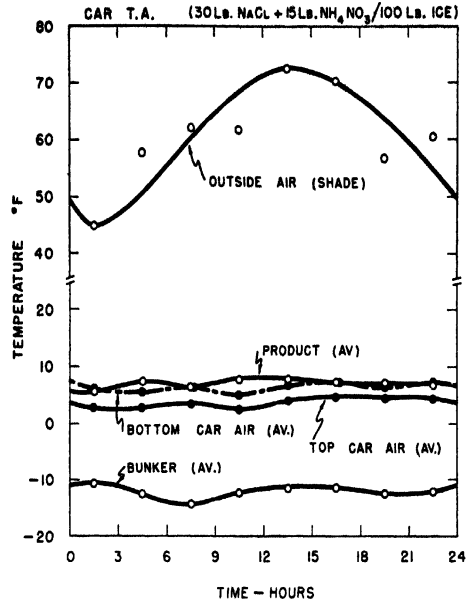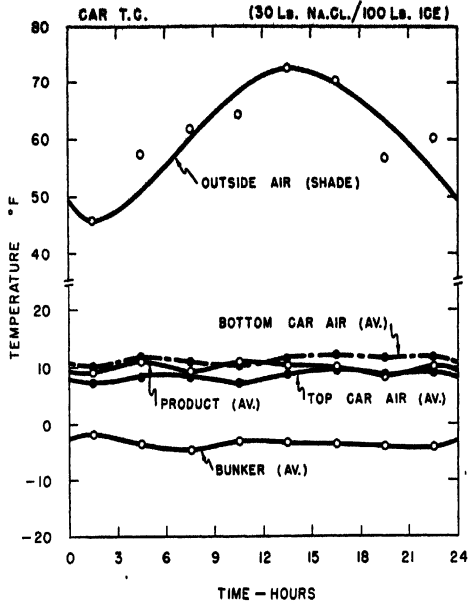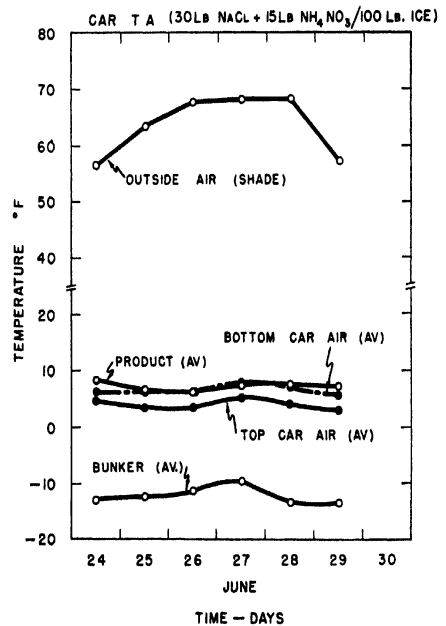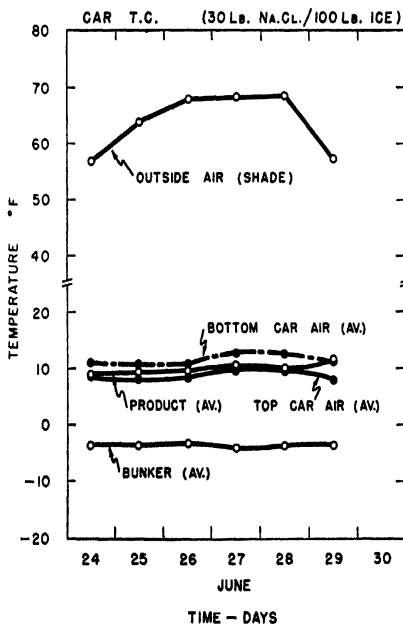
Fig. 1. Average temperature within and between days. Top air temperature was consistently cooler than the bottom air temperature attributed to tight packing of load.

100 lb); and for ammonium nitrate-NaCl-water ice mixtures, —15 F (45 to 100 lb). In other words, the mixture of both salts produces a lower melting point than can be obtained by using either salt alone.

The first question is cost. About 15 percent more total ice is required, reicings must be more frequent (at least daily), the ice must be finely crushed and thoroughly mixed with the nitrate. One large Canadian railroad estimates the increased total icing cost at 80 percent over present practice. With ice platform labor sometimes inefficient there is some question as to whether the proper proportions of the three components of the mixture would actually be thoroughly mixed in all 8 to 10 hatches of the overhead bunker type of car.

The inherent inflammable or, under certain conditions, explosive qualities of ammonium nitrate would require much greater care in handling, transportation, and storage than is required with common rock salt. Remember Texas City!

The greater acidity and corrosiveness of the ammonium nitrate brine would possibly add to the expense of maintaining the steel parts of refrigerator cars. For example, solutions of ammonium nitrate, when transported in tank cars, require special "passivation" of the steel lining by an electrolytic process, and a rubber base paint is used to coat the upper part of such tanks. Dr. Cook has himself stated that the Canadian railroads must keep their investment in refrigerator cars low and I know the corrosion effect from ordinary NaCl brine is a serious problem to these railroads, particularly in the sidewall ducts.

The future use for ammonium nitrate seems to be limited to the overhead tank type of refrigerator car and to a few types of frozen foods such as fish. In the authors' field tests, the commodity temperature at time of loading varied from 4 to 18 F. It is well known by the private carline people here in the States that such temperatures vary tremendously in actual practice, with the many different frozen food shipments and many different types of storages. Are we not perhaps considering an ultra-refinement in attempting to lower the average transit carrying temperature only 3 to 4 degrees in view of the much greater effect of variations in loading temperature?

Ordinary salt and ice will carry frozen foods successfully at 10 to 12 F car temperatures. It is adaptable to the 90 percent of perishables which do not require such low temperatures as frozen fish. It is adaptable to end bunker cars as well as overhead tank cars. The NaCl does not go into solution too fast, as does ammonium nitrate, and the refrigeration effect is longer lasting and more uniform. NaCl does not go "down the drain" so fast. Ammonium nitrate has no advantage for precooling.

By next year there will be at least 30,000 fan cars in service on the U. S. railroads. Extensive trials by the leading private carlines have demonstrated that these postwar modern cars with 4 to 4½ in. of insulation are successfully carrying frozen foods, formerly thought to be safe only in superinsulated "giant" cars. Fans, with standby motors, are most useful in precooling. Even the overhead bunker advocates are now considering the use of fans to supplement and speed up the gravity refrigeration of Canadian cars.

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Everyone concerned with the long distance shipment of frozen foods is interested in the possibilities of obtaining lower temperatures in refrigerator cars than are obtainable by use of the conventional method of mixing sodium chloride salt with ice. The results reported by Authors Carbert and Cook should therefore be scrutinized very carefully for their possible commercial application.

In their first paper (March, 1948, REFRIGERATING ENGI-NEERING) the authors point out that the mixing action produced by the motion of the car is essential for the maintenance of minimum temperatures with all salt-ice mixtures. They also found that, as compared with straight salt-ice mixtures, adding ammonium nitrate to the salt-ice mixture resulted in a further reduction of about 7 deg F in the bunker air temperature and of about 5 deg in the loading space air temperature. The additional cost was estimated at 50%.

As applied to the transportation of frozen food in the United States the additional cost need not be considered a limiting factor. Shippers would be willing to stand additional cost for the insurance of lower product temperatures. But the practical difficulty in applying the method in the United States comes from the fact that we have less than 100 cars equipped with overhead ice bunkers and not all of them have brine tanks. Practically all of our brine tank cars are used for shipping packing house products. They have the brine tanks in the ends of the cars instead of overhead. They are quite satisfactory for that use but, as is well known, they do not produce as low or uniform temperatures through the load as do overhead brine tanks, and are not suitable for frozen foods.

All of our car lines are interested in getting the frozen food business and some of them are experimenting with special types of cars designed especially to provide the low temperatures desired by the frozen food industry. Some of these experimental cars are cooled by mechanical refrigerating units, some by the absorption system of refrigeration, others by utilizing dry ice in various ways. It is too soon to say which will prove to be most acceptable. It seems safe to say, however, that overhead brine tank cars are not likely to be widely adopted in the United States or used here as general purpose cars to the same extent as they are in Canada. This is because they do not fit the varied needs of our shippers.

It does not seem likely therefore that use of special mixtures of ice and various salts to produce lower temperatures will find wide application to the frozen food industry in the United States unless it can be demonstrated that it is practicable to use such mixtures in basket bunkers with which most of our general purpose refrigerator cars are equipped. Tests along this line would be of considerable interest.

MEASURING THE SOLIDS CONTENT OF HONEY AND OF STRAWBERRY JAM WITH A HAND REFRACTOMETER[1]

BY JESSE A. PEARCE[2] AND SUZANNE JEGARD[2]

Abstract

A hand refractometer may be useful for routine inspection of the solids content of honey, and of strawberry jam containing added pectin. For 76 pairs of determinations on honey,

$$y = 1.116x - 7.58,$$

with a standard error of prediction of $\pm 0.4\%$; for 116 pairs of determinations on the jam,

$$y = 1.022x - 0.79,$$

with a standard error of prediction of $\pm 0.3\%$, where y is the per cent solids by the A.O.A.C. vacuum-oven method and x is the reading on the hand refractometer. A standard error of $\pm 0.5\%$ was observed for determinations by the vacuum-oven method, and of $\pm 0.4\%$ for determinations with the hand refractometer.

Introduction

The solids content of products such as honey and jam can be readily determined by the vacuum-oven method (1, p. 582) or with an Abbé refractometer (2). While neither method is suitable for field inspection, determinations using a hydrometer (3) have been reasonably satisfactory for inspection work. Since relatively inexpensive hand refractometers have provided a simple and rapid method of determining egg solids under field conditions (4), these laboratories were asked to calibrate a hand refractometer in terms of the solids content of honey, and of strawberry jam containing added pectin. This paper describes these calibrations.

The Hand Refractometer

The hand refractometer used in this work was manufactured by the Bausch and Lomb Optical Company, Rochester, New York. It is calibrated for sucrose solutions containing from 40 to 85% solids, and the scale can be read within $\pm 0.2\%$ at solids contents of about 40% and within $\pm 0.1\%$ at 80%. The refractometer is designed for use at 68° F. (20° C.) and has a correction thermometer attached, which is calibrated in per cent sucrose according to the temperature coefficient for the refractive index of sucrose solutions.

Application to Honey

Since the temperature coefficient for the refractive index of sucrose solutions differs slightly from that for honey (0.00018 and 0.00023 respectively per degree Centigrade), readings on honeys of different solids content were compared at temperatures of 40°, 60°, 80°, and 100° F. (4°, 16°, 27°, and 38° C.). Table I shows that the correction thermometer on the instrument can be used with reasonable accuracy over this temperature range; but that vague lines, dividing the light and dark fields in the refractometer, interfered with the

TABLE I

VARIATION IN HAND REFRACTOMETER READINGS, %, WITH TEMPERATURE OF HONEY

Sample	Solids, at various temperatures, °F.			
	40	60	80	100♀
1	♀ ♀	♀ ♀	♀ ♀	86.1
2	♀ ♀	♀ ♀	86.4	86.1
3	♀ ♀	85.0	85.0	84.8
4	♀ ♀	84.3	84.2	84.1
5	♀ ♀	83.8	83.8	83.6
6	82.0	82.1	82.1	81.9
7	81.4	81.6	81.3	81.4
8	79.1	79.1	79.0	79.1
9	78.6	79.5	79.3	77 7
10	77.0	77.2 .	77.1	77 2
11	76.7	76.7	76.4	76.6

♀ *Readings difficult, double line formed in refractometer.*
♀ ♀ *Line in refractometer too vague to permit a valid reading.*

measurement at 100° F. Temperatures between about 60° and 80° F. gave the best results and all further measurements on honey were made within these limits.

Vague lines observed for concentrated honeys held at low temperatures were attributed to partial crystallization of the sugars. Comparative measurements on samples of fluid and crystalline honeys containing 75 to 80% solids showed that crystallized honey gave hazy lines in the refractometer and could not be used for this test. All further work was done on fluid honey.

Tables relating solids content of honey by the A.O.A.C. vacuum-oven method and refractive index on an Abbé refractometer have been published (2). These data were converted to readings on the hand refractometer at 68° F. to give the curve shown in Fig. 1. Honey was diluted to give a range of solids from 76 to 88%, determined by the vacuum-oven method, and the mixtures were examined in the hand refractometer. The results are given as points on Fig. 1; they show close agreement between the previous and present techniques, and support the use of the hand refractometer.

To assess the application of this instrument, the solids in 76 samples of honey obtained in routine inspections were determined by the vacuum-oven method and with the hand refractometer.   The samples were obtained during
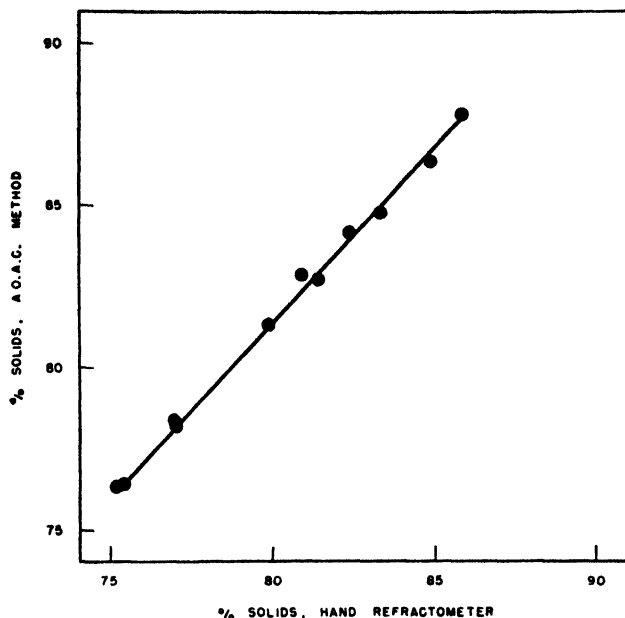

Fig. 1.   *Relation between solids in honey as determined by the A.O.A.C. vacuum-oven method and by the hand refractometer.*
Solid line—values converted from refractive indices in Ref. (2).
Points—values determined in course of present study.

1946 and 1947 from all Canadian provinces but New Brunswick and Prince Edward Island and were classed as white, golden, light amber, dark amber, and dark.   Statistical analysis showed that, in general, the color of the honey and the year of production had little effect on the measurement, but that honey of the same color from different provinces gave equations that were significantly different.   For example, the regression lines for golden honey from different provinces, shown in Fig. 2, are statistically different, but the maximum difference between the curves is about the same order as the standard error ($\pm 0.4\%$) of an equation for 76 samples,

$$y = 1.116x - 7.58$$

where y is the per cent solids by the vacuum-oven method and x is the hand refractometer reading.

Application to Strawberry Jam Containing Added Pectin

The solids in fluid honey can be determined without preliminary separation, but the solids in jams are usually determined on a separated fluid that is as free as possible from suspended matter.   In this work, separation was effected

by straining juice from the jam through muslin bolting cloth No. 12XX
(1, p. 381).   The solids in the strained liquid were determined by the hand
refractometer, and by the A.O.A.C. vacuum-oven procedure (1, p. 556),
but using sand in the moisture tins (1, p. 557).

Although the solid material in fluid separated from jam is primarily sucrose,
the correction thermometer on the hand refractometer was checked by readings
on four jams, ranging in solids from 62 to 71%, at temperatures of 40°, 60°,
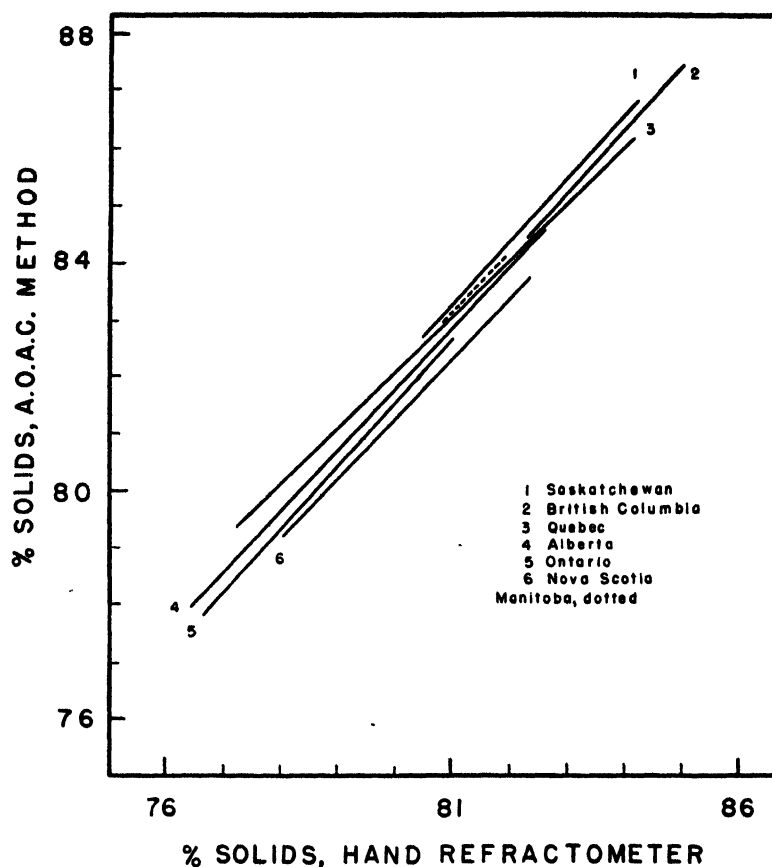
FIG. 2.   *Relation between solids in golden honeys, by provinces, as determined by A.O.A.C.*
vacuum-oven method and by the hand refractometer.

80°, and 100° F.   The averages were 66.8, 66.6, 66.3, and 67.0% at the four
respective temperatures, with a necessary difference of 0.4%*.   Consequently,
a temperature range from 60° to 80° F. is recommended when using the hand
refractometer for inspection work.

To determine the error in either method and the relation between the two
methods of measurement, 29 one-pound samples of strawberry jam with added

* *Five per cent level of statistical significance.*

pectin were collected during routine inspections, and each sample was divided into four parts, which were placed in sealed containers. Single measurements of the solids content were made on different days on each part of each sample by both techniques.

For the 116 pairs of measurements on these jams, which ranged from 61% to 72% in solids, the relation between the per cent solids by vacuum-oven method (y) and the hand refractometer reading (x) was

$$y = 1.022x - 0.79,$$

with a standard error of prediction of $\pm 0.3\%$.

The vacuum-oven method, which is usually considered to be the reference method, had no greater accuracy than the hand refractometer. An examination of the data for jams showed that solids contents were determinable by the vacuum-oven method with a standard error of $\pm 0.5\%$, and by the hand refractometer with a standard error of $\pm 0.4\%$.

Acknowledgments

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Changes in the Palatability of Cod Fillets

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ABSTRACT

A comparison has been made of the assessment of quality by taste panels and by the level of trimethylamine in cod fillets stored at 0° C. and 5° C. The correlation was good, indicating that a trimethylamine level of about 15 mg. N per 100 g. tissue is on the borderline between acceptance and rejection. There is no ripening process as in meat, the quality falling off before there is chemical evidence of spoilage.

Most foods undergo chemical and physical changes unless they are adequately protected from bacterial and enzyme action by drying, freezing, sterilizing or some other method of preservation. These changes may improve the palatability of the food as the aging of meat improves the flavour and texture, or they may cause spoilage flavours and odours and undesirable changes in the texture. Such changes can be followed by chemical, bacteriological and physical measurements but the *desirability* of any change in a food can only be measured subjectively. For this reason the taste panel technique has come into use. It has been found valuable in studying the palatability of a wide variety of foods, including roast beef (Cover 1936; Paul, Lowe and McClurg 1944), oranges (Harding and Wadley 1945), meat scrapple (Carl, Watts and Morgan 1944), turnip greens (Whitacre *et al.* 1944), chicken (Stewart *et al.* 1945), roast pork (Hardy and Noble 1945) and many others. Platt (1937) has discussed the taste panel method and Dove (1946a, 1946b, 1947) has described the use of this technique in extensive studies of food acceptance and rejection by the American soldier-consumer.

In the work reported here we have used taste panels to determine the effect on the palatability of cod fillets of changes which occur during storage, and the correlation between consumer acceptance and spoilage levels.

Previous workers have described the course of spoilage in cod and haddock muscle (Beatty and Gibbons 1937; Beatty and Collins 1939; Collins 1941; Dyer and Mounsey 1945). During the struggling of the fish when caught and immediately after its death the glycogen of the muscle is converted to lactic acid. When the fish is held in storage, spoilage bacteria convert the lactic acid to volatile acids which may be detected organoleptically by a sweet or sour smell from the raw fish. At the same time the trimethylamine oxide in the muscle is

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reduced by the bacteria to trimethylamine, the substance which is mainly responsible for the fishy smell in spoiling sea fish. It is not until spoilage is well advanced and trimethylamine has increased to approximately 60 mg. N. per 100 g. that any appreciable amount of proteolysis takes place with the production of putrid-smelling amines and other smelly products of protein decomposition.

The trimethylamine test, which may be used to measure the spoilage before the fish first becomes objectionable and until it is no longer fit for consumption, is the best measure of spoilage in this type of fish (Beatty and Gibbons 1937; Beatty and Collins 1939; Dyer and Mounsey 1945). In the present work spoilage has been determined by the trimethylamine test (Dyer 1945) and by organoleptic examination of the raw fish. The two chief factors affecting the spoilage of fish under commercial conditions are length and temperature of storage. Accordingly the effect of these factors on the palatability of cod fillets has been measured.

STORAGE OF FILLETS

Two storage temperatures were used, 0° C., equivalent to the best commercial practice, and 5° C., approximately the temperature of the household refrigerator. Very fresh fish still in rigor were used as starting material. They were filleted, washed, and the fillets doubly wrapped in parchment paper to prevent drying. They were stored in waxed cartons at 0° C., by keeping them buried in crushed ice in a room at 3 to 6°, or at 5° C. in a constant-temperature cabinet.

The fillets were put in storage on successive days so that for any taste panel there were available fillets of three ages from 1 to 15 days at 0°, and from 1 to 5 days at 5° C., with a fresh control. Usually fillets of a particular age were judged by five or six taste panels all on different days so that there were as many different lots of fish as there were taste panels.

TASTE PANEL PROCEDURE

Seventeen members of the staff acted as taste panel judges in the experiment at 0°, and twelve in the run at 5° C. They were selected from twenty-four persons available, according to their ability to discriminate between fresh and stale fish in a preliminary series of taste panels. They were not required to serve oftener than once in the morning and once in the afternoon of any one day. As far as possible, ten judges were used on each panel.

All the samples of a particular age for each taste panel were cut from the thick part of a single fillet so that there was as little variation in the samples as possible. A small piece was cut from each sample to make a composite sample for trimethylamine analysis. The pieces, approximately $2'' \times 1\frac{1}{2}'' \times \frac{7}{8}''$ (1 in. = 2.5 cm.), were placed in numbered rows in aluminum pans so that each judge's samples came from a single pan to eliminate possible differences in the temperature of the different parts of the oven. The pans were very lightly greased with cottonseed oil and the fish baked at 550° F. (290° C.) for 11 minutes. No salt was used. They were served in numbered petri plates kept warm during

TABLE I. Score card used in the taste panels.

| Name of judge | | | | | Sample no. | | Date | | |

Surface appearance	No. 1	No. 2	No. 3	No. 4	Odour	No. 1	No. 2	No. 3	No. 4
0 { not flaked open / firm / moist / translucent					0 Fresh fish odour				
1 white					1 no odour				
3 { yellow / flaked open / dry / soft / wet					2 sweet (volatile acid) / sour				
5 red brown				•	3 fishy (trimethyl-amine)				
					4 stale (enough tri-methylamine to be objectionable)				
					5 rancid / putrid				
Remarks:					Remarks:				

Texture	No. 1	No. 2	No. 3	No. 4	Taste	No. 1	No. 2	No. 3	No. 4
0 { firm / tender / flakes well / moist					0 sca-fresh tang				
2 { watery / soft / mushy / mealy / not flaky					1 more or tess taste-less				
4 { rubbery / leathery / stringy / tough / coarse					2 sweet / sour				
5 { hard / dry					3 slightly fishy (tri-methylamine)				
					4 stale (enough tri-methylamine to be objectionable)				
					5 rancid / putrid				
Remarks:					Remarks:				

Freshness	No. 1	No. 2	No. 3	No. 4	Grade	No. 1	No. 2	No. 3	No. 4
0 just caught					0 perfect				
1 fresh					1 good				
3 not very fresh					2 fair				
4 spoiling					3 borderline				
5 spoiled					4 spoiling				
					5 spoiled				
Remarks:					Remarks:				

TABLE II. Taste-panel ratings of cod fillets stored at 0° C. in relation to days of storage and to amounts of trimethylamine (TMA = mg. N per 100 g.)

	As per cent of judgements at each age														
Days of storage	0	1	2	3	4	5	6	7	8	9	10	11	12	13	15
Number of judgements	104	90	31	56	38	40	49	37	26	44	51	53	58	23	6
Odour							Per cent								
0	30	24	20	16	13	13	16	6	13	7	9	4	8	0	0
1	26	47	40	45	34	34	31	34	8	40	28	9	21	9	17
2	15	13	17	14	29	34	31	24	29	28	26	29	17	10	17
3	14	14	23	20	10	13	14	12	21	14	16	11	8	24	0
4	10	2	0	5	11	6	6	12	29	11	21	21	23	38	33
5	5	0	0	0	3	0	2	12	0	0	0	26	23	19	33
Taste															
0	30	27	0	10	3	15	10	10	4	9	5	2	3	0	0
1	12	35	63	48	44	35	45	40	32	51	38	17	27	5	14
2	27	24	22	18	20	33	35	18	18	23	23	14	13	14	43
3	19	12	16	15	10	15	8	13	7	14	12	19	13	9	29
4	10	2	0	7	18	2	2	16	39	2	20	19	22	50	14
5	1	0	0	0	5	0	0	3	0	0	2	29	22	23	0
Grade															
0	30	24	10	7	5	10	6	5	4	2	10				
1	23	36	32	33	21	22	29	16	15	32	20	4	14	4	
2	15	22	40	32	21	38	43	35	19	43	28	15	10	13	17
3	12	12	12	18	31	25	18	30	8	16	24	19	26	9	17
4	14	3	3	7	8	5	2	5	35	7	12	28	19	26	17
5	6	2	0	2	13	0	2	8	19	0	8	34	31	44	50

the judging on special copper boxes 14″ × 14″ × 3″ filled with hot water. The copper boxes were large enough to allow a space of more than 6″ between the petri plates to minimize mingling of odours from the samples.

The opinions of the judges were recorded on score cards (table I) on which they checked the appropriate terms descriptive of the surface appearance, texture, odour, taste and freshness, and graded the samples from perfect to spoiled. For statistical purposes each descriptive term or group of terms representing a particular stage of fish spoilage, such as volatile-acid production, trimethylamine oxide reduction and protein decomposition, was given a numerical value of 0 to 5 (table I). In calculating the results these numerical values were expressed as per cent, 0 = 100%, 1 = 80%, 2 = 60%, 3 = 40%, 4 = 20%, 5 = 0%. Fish graded perfect was thus given a rating of 100%, that graded good 80%, fair 60%, borderline 40%, spoiling 20% and spoiled 0%.

In practice it was found that the surface appearance and the texture ratings were of little significance in the present experiment, and the ratings for freshness were almost always identical with the grade. The discussion of the results has been confined, therefore, to the ratings for odour, taste and grade.

TABLE II (continued)

	As per cent of judgements at each TMA value																	
TMA of fillet	0	1-2	3.5	4	5	5-6	7-8	10-11	14	16-17	21	24-25	26-27	29	31	34	35-36	39-40
Number of judgements	363	46	12	16	15	17	25	19	36	21	8	20	17	9	20	18	11	18
Odour							Per cent											
0	21	8	25	6	0	8	9	0	0	5	11	5	0	0	0	11	9	6
1	38	40	33	22	44	23	36	56	3	10	33	16	44	0	14	11	27	6
2	18	48	25	5	25	30	32	22	40	20	33	26	22	14	18	17	0	12
3	15	4	9	28	12	8	18	17	20	10	22	16	17	0	14	0	0	23
4	7	0	8	39	19	23	4	5	23	25	12	16	17	43	32	33	9	30
5	1	0	0	0	0	8	0	0	14	30	0	21	0	43	22	28	55	23
Taste																		
0	17	17	16	5	0	25	13	5	0	10	0	0	0	0	0	0	11	0
1	35	47	43	37	27	31	50	55	22	15	50	21	40	0	9	0	11	26
2	26	26	16	0	33	19	25	25	27	10	25	11	20	14	22	17	6	10
3	15	8	25	16	7	13	8	15	11	5	12	31	27	0	13	0	0	16
4	7	2	0	42	20	0	0	0	35	25	13	21	13	14	39	17	50	22
5	0	0	0	0	13	12	4	0	5	35	0	16	0	72	17	66	22	26
Grade																		
0	14	15	8	6	0	12	4	0	0	0	13	0	6	0	0	0	0	0
1	25	35	25	19	33	12	20	32	17	14	25	5	12	0	0	6	0	17
2	32	17	50	19	27	41	48	42	22	14	25	20	29	22	5	0	0	0
3	16	30	8	6	13	18	20	21	31	14	13	15	35	11	30	28	18	11
4	7	2	8	31	7	5	4	5	22	33	25	45	18	0	20	28	9	28
5	4	0	0	19	20	12	4	0	8	24	0	15	0	67	45	39	73	44

CHANGES IN PALATABILITY AT 0° C.

As would be expected in an experiment depending on the opinions of human judges for data, there was a good deal of variation in the results (tables II and III). The clearest picture of the results is obtained from smoothed curves which avoid the confusion caused by these variations in judgment. In the curve for 0° C. (fig. 1), the points plotted are the averages for each 2 days, while in figure 2 the average grades are plotted for increments of 1 in trimethylamine values.

In the fillets stored at 0° C. (fig. 1) the trimethylamine increased gradually during the first week of storage and then increased very rapidly during the second week. At the same time the taste-panel grades decreased gradually during the first week, and rapidly from 8 to 15 days as the fish became spoiled. The average grade given the fillets at 0° C. fell off gradually from 67% at the beginning of storage to 52% at 8 days, and then dropped rapidly to 20% and lower at the end of 14-days' storage. Thus fish which had been stored a week were graded as from borderline to fair, at 12 days mostly as spoiling and after 12 days

as spoiled and spoiling. At the same time trimethylamine increased gradually from less than 1 mg. N per 100 g. at 0 days to 4 to 14 mg. at 8 days, and then increased rapidly to 30 to 40 mg. N per 100 g. at 12 and 13 days.

The palatability of the fillets steadily decreased with increasing trimethylamine. The highest palatability ratings were given to fillets with a trimethylamine value of less than 2 to 4 (fig. 2), a trimethylamine value of 15 was borderline between fresh and spoiled, and fish with a trimethylamine value of 15 to 30 and over were mostly rated as spoiling and spoiled.

In figure 2, calculation of the relation between grade and trimethylamine value by the Method of Least Squares gave the equation, grade = 62 − 1.19 (trimethylamine value) for the samples stored at 0° C. The regression coefficient (r) was 0.71, showing a fair degree of correlation.

The taste panel results showed that not only did palatability decrease as trimethylamine increased but also that the palatability was lowered with in-

TABLE III.   Taste-panel ratings of cod fillets stored at 5° C., in relation to

As per cent of judgements at each age					
Days of storage	0	1	2	3	4	5
Number of judgements	30	30	40	40	30	10
Odour	Per cent					
0	33	14	8	10	3	
1	47	43	42	31	7	
2	13	18	21	23	13	
3	7	21	10	18	7	30
4		4	16	18	27	30
5			3		43	40
Taste						
0	24	3	5	7		
1	41	57	36	26	7	
2	21	27	28	15	7	11
3	14	13	7	26	23	11
4			23	21	13	11
5				5	50	67
Grade						
0	23	3	10	7		
1	50	23	5	21	7	
2	13	53	38	13	3	10
3	7	20	25	23	17	10
4	7		15	21	13	20
5			7	15	60	60

creasing age. The quality of cod fillets was definitely lowered by even a short storage period at 0° C. In this, cod fillets differ from meat, which is improved by aging. The fresher the fish the more palatable it was, and even before spoilage could be detected by organoleptic examination of the raw fish and before the trimethylamine content had started to increase, the palatability was reduced.

In general the numerical ratings for odour, taste and grade of the fillets stored at 0° C. were very closely related, so that the ratings describing each spoilage stage made parallel curves when plotted and have been drawn as one line for simplicity (fig. 3). In this graph smoothed curves only have been plotted, the points being omitted for clarity. The freshest fish was given the highest palatability rating. At 0 days (fig. 3) 30% of the fillets were rated as perfectly fresh (fresh fish odour, sea-fresh taste, perfect grade), but the number of samples given these high palatability ratings decreased rapidly to 10% at 5 days and none at 12 days. At 0 days, 20% of the samples were also rated as fresh or good fish (odourless, more or less tasteless, good grade) and this increased to 43% at 1 and 2 days as fewer samples were rated perfect and then gradually decreased to 15% at 12 days.

From 3 to 8 days sweet or sour odours and tastes were predominant, and 30 to 35% of the samples were rated as sweet or sour, 30 to 35% were graded

days of storage and to amounts of trimethylamine (TMA = mg. N per 100g.)

	As per cent of judgements at each TMA value										
TMA of fillets	0	1	2	2.5	9	10	13	17	31	52	62
Number of judgements	40	30	20	10	10	20	10	10	10	10	10
Odour					Per cent						
0	28	10	11	11	20	15	10	10			
1	51	35	47	22	30	40	20	60			
2	13	28	21	33	10	20	30	20			10
3	8	21	10	22	20	15	20	10		30	
4		7	10	11	20	10	20		30	30	20
5									70	40	70
Taste											
0	20	4		20	10						
1	40	51	39	20	20	15	30	30			
2	30	31	39	30	10	10	10	40		10	
3	10	7	11	20	20	35	10	30		30	20
4		7	11	10	20	35	40		20	20	10
5					20	15	10		80	40	70
Grade											
0	21		21	10	10	5					
1	44	17		30		10		50			
2	23	55	48	20	10	5	10	20		10	
3	10	24	21	30	20	30	30	30	10	10	10
4	2	4	5	10	40	25	40			20	90
5			5		20	25	20		90	60	

fair, and 20 to 30% as on the borderline between fresh and spoiled.

At 8 days stale and even putrid tastes and odours were noticeable. Fifteen % were rated as stale and spoiling at 8 days, 25% at 11 days and 40% at 13 days. The samples rated putrid and spoiled increased from 8% at 8 days to 15% at 11 days and 30% at 13 days.
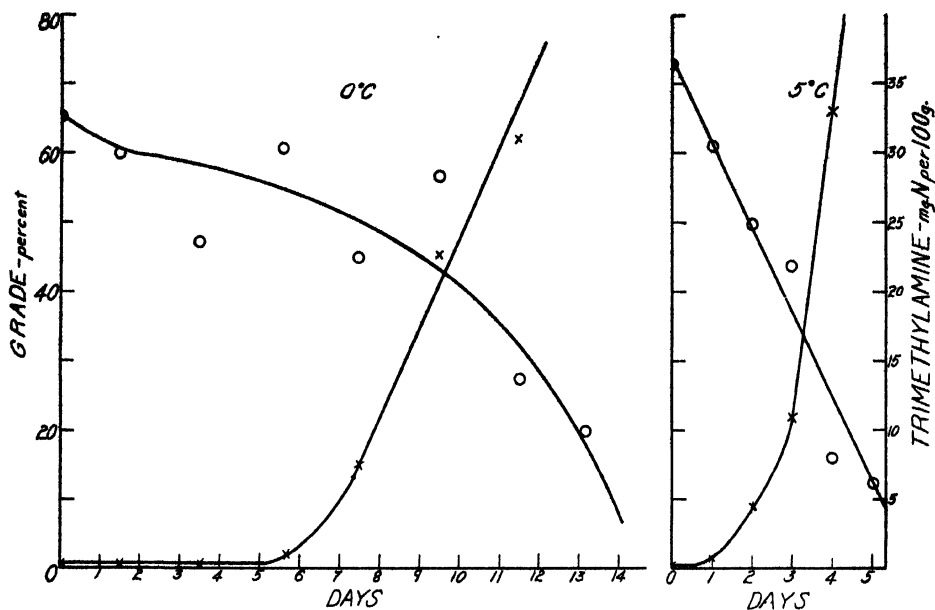
FIGURE 1. Taste panel scores, as average points for each 2 days fish samples, and trimethylamine values, for cod fillets stored at 0° C. and at 5° C. Taste panel scores, 0; trimethylamine values, X.

Approximately 15% of the samples were rated as fishy smelling and tasting all through the experiment. This is probably partly due to a certain amount of confusion among the judges as to what constituted a fishy smell, and partly to the presence of other odours.
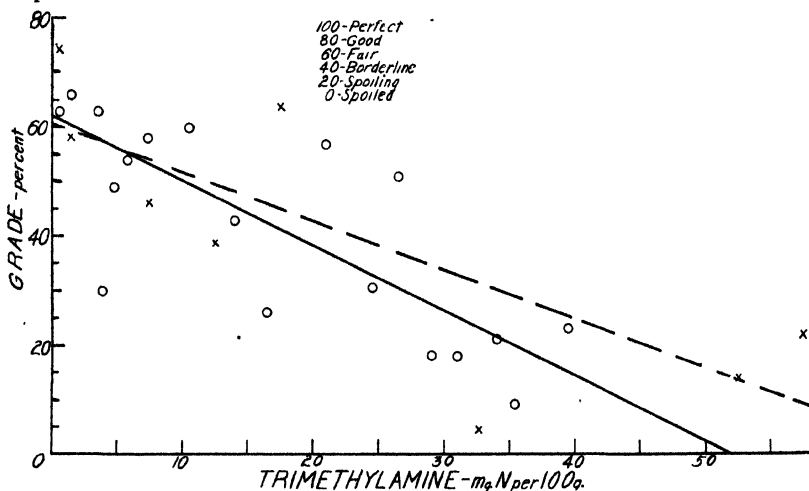

FIGURE 2. Relationship between grade (per cent) and trimethylamine values for cod fillets stored 0 to 13 days at 0° and 5° C. 0 stored at 0° C.—regression equation at 0° C., grade = 62 —1.19 (trimethylamine value); X stored at 5° C.—regression equation at 5° C., grade = 60 —0.89 (trimethylamine value).

The fillets stored 8 days and longer at 0° C. were graded spoiled when examined organoleptically before cooking. This corresponds with the first appearance of the more objectionable spoilage flavours and odours in the cooked fish, trimethylamine values of about 15 and taste panel grades of spoiling and spoiled. This may be compared with previous spoilage levels of 10 mg. trimethylamine N per 100 g. (Beatty and Gibbons 1937), 10 to 20 mg. trimethylamine N per 100 g. (Dyer and Mounsey 1945) and 15 mg. trimethylamine N per 100 g. (Dyer and Dyer 1947), which were chiefly obtained by correlation of the trimethylamine content with the odour and appearance of the raw fillets.
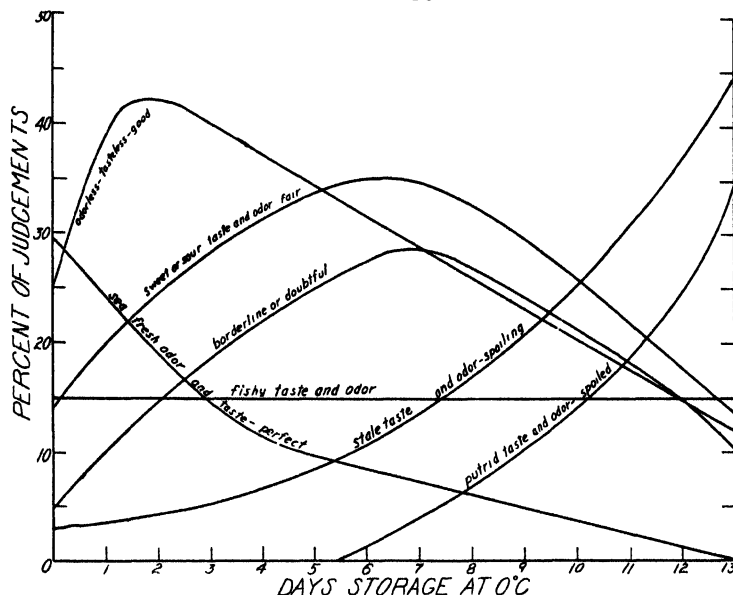
FIGURE 3. Succession of odours and tastes (smoothed curves) as per cent of the number of judgements of each day's samples for cod fillets stored 0 to 13 days at 0° C.

The taste panel results showed that there was a definite series of changes in the palatability of the stored cod fillets. When less than 1 day in storage the fillets had a delicious fresh-caught flavour and odour. This rapidly disappeared, leaving the fish odourless and tasteless. Then, as spoilage began, sweet-sour and fishy odours and tastes became noticeable and finally putrid odours and tastes made the fish very unpalatable.

While the results with cod fillets showed that the freshest fish were considered best by the taste panel, this is probably not the case for all species of fish.

CHANGES IN PALATABILITY AT 5° C.

A small number of fillets were stored at 5° C. from 0 to 5 days. The palatability changes at this temperature were similar to the changes in the fillets stored at 0° C. except that spoilage was much faster (fig. 1).

The freshest fish were given the highest palatability rating. At 0 days one-quarter of the samples were given the highest possible taste and odour ratings

(fresh fish odour, sea-fresh taste) and one-third were graded perfect. At the same time 40-50% of the samples were rated odourless, more or less tasteless and graded good.

The palatability was already starting to decrease after one day's storage at 5° C. Only 14% of the samples were rated as fresh smelling, and only 3% were rated sea-fresh tasting and graded perfect. Forty-three % were rated odourless and 57% tasteless. Twenty-three % were graded good, 53% fair and 20% borderline after one day at 5° C.

Sweet-sour odours and tastes were not particularly noticeable in the fillets stored at 5° C., probably because spoilage was so rapid that the stronger spoilage odours and tastes appeared very soon after the odourless and tasteless stage. It is possible that the stage of volatile-acid spoilage would have been detected if a larger number of samples had been used. From 13 to 23% of the samples were rated sweet or sour smelling from 0 to 4 days and none at 5 days. Fifteen to 28% were rated sweet or sour tasting from 0 to 3 days and 7 and 11% at 4 and 5 days.

Seven to 30% of the samples were rated fishy smelling and tasting all through the storage period.

Stale odours and tastes began to show up after 2 days' storage at 5° C. At 2 and 3 days only 5 to 10% of the samples were given the sea-fresh taste and sea-fresh odour ratings and graded perfect. Forty-two and 31% were rated odourless and 36 and 26% tasteless at 2 and 3 days. Ten and 7% were graded perfect, 5 and 21% good, 38 and 13% fair, 25 and 23% borderline, 15 and 21% spoiling, 7 and 15% spoiled at 2 and 3 days.

At 4 and 5 days the fish were definitely of poor quality. Twenty-seven to 30% were rated as stale smelling and 40% as putrid smelling. Fifty to 67% were rated as putrid tasting and 60% were graded spoiled.

The trimethylamine content of the fillets stored at 5° C. increased from less than 1 mg. N per 100 g. at 0 days to 7 to 17 mg. at 3 days and at 4 days averaged 32, with some samples as high as 60 mg. N per 100 g. As the tri-methylamine content increased, the palatability decreased rapidly (fig. 1). The average grades for the fillets stored at 5° C. were good (74%) at 0 days, fair (62%) at 1 day, borderline at 2 days (49%), and 3 days (45%), spoiling and spoiled at 4 days (17%) and 5 days (14%).

Here the regression equation was, grade = 60.3 − 0.89 (trimethylamine value), with a regression coefficient of 0.88. Thus at 5° C. there is an even better correlation than at 0° C. This would indicate that at 5° C. the trimethyl-amine value contributed relatively more than at 0° C. to the taste panel rating than the other spoilage products.

CORRELATION WITH SURVEYS OF QUALITY IN RETAIL STORES

Surveys of the quality of cod and haddock in the retail stores in the Maritime provinces and central Canada have shown that the fish is often not fresh when offered to the consumer (Beatty *et al.* 1936, 1937, unpub., Dyer *et al.* 1943, unpub.). In these surveys the quality of the fish was determined by the tri-

methylamine content and by organoleptic examination of the raw fish. Since we have correlated the taste panel results with these same measures of spoilage, the results of the quality surveys may be compared with the taste-panel findings.

One retail survey conducted in Toronto in summer showed 44% of the samples with a trimethylamine content of 0 to 4 mg. N per 100 g., 38% with a trimethylamine value of 4 to 15 and 18% with more than 15 mg. trimethylamine N per 100 g. Thus, 44% would be good fish, 38% would be in the early stages of spoilage when sweet-sour odours and tastes are predominant, and stale and putrid tastes and odours are becoming noticeable, while in 18% stale and putrid tastes would predominate and the fish would be graded spoiling and spoiled by our taste panels.

The quality of the fish in the Montreal retail stores was very similar, although the fish in the Montreal wholesale stores was much better. Here 80% of the fish were good (trimethylamine of less than 4) and 20% borderline (trimethylamine of 4 to 15) with almost no spoiled fish.

The quality surveys have shown that the Halifax retail market offers its consumers fish of better quality than is available inland. This probably reflects the nearness of the retail market to the producers. Here the fish goes directly from the fish plants to the retail stores so that there is no long rail trip and no storage in wholesale stores as there is inland. In two surveys made six years apart approximately 60% of the fish had a trimethylamine value of 0 to 4, 27% had a trimethylamine value of 4 to 15, and only 13% had a trimethylamine content of more than 15 mg. N per 100 g. Thus, 60% of the fish offered the Halifax consumers were fresh. Twenty-seven % were in the borderline stage between fresh and spoiled, where sweet-sour odours and tastes are predominant and more objectionable spoilage odours and flavours start to become noticeable in the cooked fish. Thirteen % were spoiled with putrid tastes and odours, making the fish unpalatable.

Thus, it is obvious that the quality of the fish reaching the inland consumer is not good. Although the quality of the fish in the Halifax retail stores is much better, even there, near the producers, the consumer is by no means certain to obtain good fish. In inland cities 40% of the fish would be considered fresh by the taste panels, although most of it would probably be more or less tasteless. Thirty to 40% would be classed as borderline with a palatability rating little better than half that of fresh fish, and the remaining 20 to 35% spoiling or spoiled.

Inland people so seldom get really fresh sea-fish that it is probable that they consider spoilage tastes and odours normal characteristics of sea food. There can be little doubt that the low quality of the fish available in the retail stores is a factor in the small consumption of fish. In 1944 consumption was only 9 lb. (1 lb. = 0.45 kg.) per person per year in Canada and 8.5 lb. in the United States compared with meat consumption of 158 lb. in Canada and 155 lb. in the United States (Jacobson, McArthur, Lewis and Williams 1944). To those familiar with the delicious flavour of fresh fish it seems obvious that consumers would prefer better fish if they could get it.

CONCLUSIONS

Aging of fish does not improve the palatability. The freshest fillets had the highest palatability rating.

Quality is steadily lowered during storage. Even before spoilage tastes and odours become noticeable the palatability is reduced. Cod fillets which had been in storage for only one day had lost much of the original sea-fresh flavour.

Under our conditions it took the fillets approximately 8 days at 0° and 3 days at 5° C. to reach the unacceptable stage. Fish with a trimethylamine value of 4 to 15 is in the early stages of spoilage when sweet-sour odours and tastes are predominant and stale and putrid tastes may be present. Such fish may or may not be considered spoiled by those eating it. Fish with a trimethylamine value of 15 to 30 and over is very likely to be considered spoiled or spoiling by the consumer who eats it, as stale and putrid tastes and odours make the fish most unpalatable.

Surveys of the quality of fish in the retail stores have shown that much of it is of such poor quality that our taste panels would rate it as borderline between fresh and spoiled or as actually spoiled.

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Bacterial Reduction of Sodium Nitrite and Formation of Trimethylamine in Fish

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ABSTRACT

Bacteria reduce sodium nitrite in stored cod fillets. Rapid reduction of trimethylamine oxide is inhibited by the nitrite in the concentrations used, up to 700 p.p.m., trimethylamine formation occurring only after the nitrite is reduced to about 50 p.p.m. This results in an increased keeping time in fillets treated with nitrite. The surface pH remains acid until the rapid trimethylamine formation takes place.

Nitrate alone, more slowly in the presence of nitrite, is rapidly reduced to nitrite and beyond. The trimethylamine oxide reduction is not affected by the nitrate reduction, the former being usually reduced before the nitrate.

Sodium nitrite was first used on fish by Taylor (1933). Its effect on bacteria in fish has been studied by Tarr in a series of publications (Tarr and Sunderland 1940; Tarr 1941, 1944). Evidence was obtained that nitrite inhibits bacterial growth particularly in acid solution. Working with cod fillets (pH 6.3 to 6.9) Castell (1949) has shown that sodium nitrite in concentrations below 200 p.p.m. has little or no inhibitive effect on bacterial growth. He found rather that trimethylamine formation is inhibited.

If the preservative action of nitrite is not due to a reduction in bacterial population, it is probably concerned with an effect of the nitrite on the enzymic reactions of the bacteria. There is a considerable body of evidence to show that bacteria reduce nitrate to nitrite and then to hydroxylamine and, according to conditions, to nitrogen or to ammonia. ZoBell (1932) emphasized that many species of bacteria bring about the reduction of nitrite in addition to the well-known reduction of nitrate, and suggested that nitrogen gas may be formed in certain cases. Wooldridge and Corbet (1940) and Corbet and Wooldridge (1940a, 1940b) have shown that nitrate may be reduced to either nitrogen or ammonia in sewage; no reduction occurred unless bacteria were present, thus showing that chemical reduction did not account for the nitrate disappearance. Lindsey and Rhines (1932) were able to detect hydroxylamine qualitatively in transient amounts during the reduction of nitrite. A most complete study of bacterial nitrate reduction is reported by Woods (1938), who showed that certain washed bacterial cells in the presence of hydrogen reduce nitrate, nitrite and hydroxylamine, absorbing amounts of hydrogen equivalent to ammonia

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formation. He showed further that the reduction of nitrite is the slowest reaction, that the reduction of nitrate to nitrite is usually faster than the above, and that the reduction of hydroxylamine is very much faster than either of the other reactions. This latter would explain the usual inability to detect hydroxylamine in the reduction system. Attempts to trap the latter by formation of oxides with ketones were unsuccessful. Woods showed also that pH affects the three reactions differently, acid conditions favouring nitrite reduction and alkaline favouring nitrate and hydroxylamine reduction. Wirth and Nord (1943) obtained positive tests for the presence of hydroxylamine during nitrite reduction and also confirmed Woods' observation that the nitrate to nitrite reaction is faster than that of nitrite to hydroxylamine, but slower than the reduction of the latter.

Tarr (1944) found that an acid medium strongly inhibits the reduction of sodium nitrate to nitrite in nutrient broth by bacteria from spoiling fish in agreement with the results of Meiklejohn (1940). Large amounts of nitrite could be formed in neutral or alkaline solution. On the other hand, he did not find sodium nitrate to be reduced when added to fish muscle.

With the above body of evidence, it appears very probable that the reduction of these nitrogen compounds follows the following path: nitrate → nitrite → hydroxylamine → either ammonia or nitrogen. Tarr (1945) showed that hydroxylamine strongly retards the growth of certain fish-spoilage bacteria. Since Woods has shown the transient nature of hydroxylamine production, and since Castell (1949) has shown that there is no inhibition of bacterial multiplication in nitrite concentrations up to 200 p.p.m., the preservative action of nitrite is probably not due to hydroxylamine.

There is considerable evidence to show that the nitrite or nitrous acid is the inhibiting agent rather than their breakdown products. Bernheim (1943) obtained some evidence that under acid but not under alkaline conditions nitrous acid combines with the amino groups of various enzymes rendering them inactive. This was first suggested by Quastel and Wooldridge (1927) who found that dehydrogenase systems are inhibited by nitrite. Sciarini and Nord (1944) showed that nitrite in acid solution inhibits the enzyme carboxylase and that the reduction of nitrate to nitrite is probably effected by two separate systems, (1) by hydrogen from dehydrogenases, a reaction which is cyanide insensitive, and (2) by a reductase (nitratase) which is cyanide sensitive (Quastel 1932). Sciarini and Nord (1945) confirmed Bernheim's findings that the combination of nitrous acid with the amino groups of carboxylase inhibits this enxyme, and they obtained evidence for the presence of the resulting diazo compound.

The above work can be summarized briefly as follows: (1) The ordinary symptoms of spoilage in fish are delayed in the presence of nitrite; (2) this is not due to inhibition of bacterial growth at low sodium nitrite concentration below 200 p.p.m. and at the normal pH of cod fillets; (3) there is considerable evidence that nitrite is reduced to nitrogen or ammonia, depending on the conditions, but this provides no explanation for the preservative action of nitrite; (4) nitrite

has an inhibiting effect on at least some of the enzyme systems normally active during the growth of spoilage bacteria.

Since trimethylamine is one of the earliest formed and most objectionable products in the bacterial spoilage of most sea fish, it is important to know the time relationship of the reduction in concentration of trimethylamine.

<center>EXPERIMENTAL</center>

EFFECT OF SODIUM NITRITE

Cod fillets containing about 240 p.p.m. sodium nitrite were wrapped in waxed paper and stored at 5° C. Samples of about 100 g. were taken daily for analysis. The sodium nitrite concentration of the samples was determined by the method
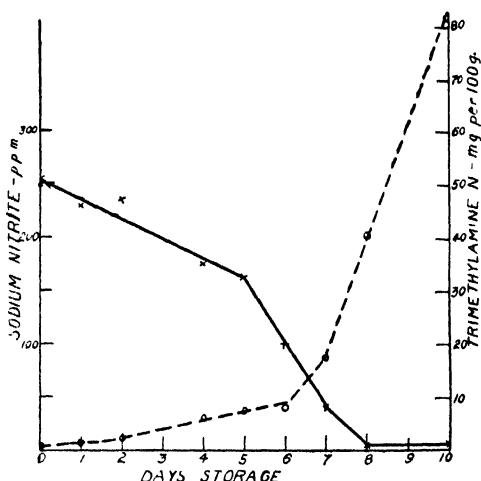
FIGURE 1. Nitrite reduction and trimethylamine increase in nitrite-treated fillets stored at 5° C. Nitrite x————x; trimethylamine o————o.

of Dyer (1946), and the trimethylamine content by the picrate method (Dyer 1945). The results are shown in fig. 1. It is evident that the nitrite begins to decrease almost immediately. This decrease becomes very rapid after 5 days and the nitrite concentration is reduced to almost 0 after 8 days. Thus, the bacteria present are able to reduce the nitrite quite rapidly. It will be shown in a subsequent paper that the reduction of nitrite is actually due to bacterial activity. During the reduction of nitrite only small amounts of trimethylamine were formed, about 8 mg. nitrogen per 100 g. fish being produced after 6 days, by which time the sodium nitrite had decreased to 100 p.p.m. After 7 days the nitrite content was about 40 p.p.m. and trimethylamine was being produced rapidly. In untreated fish stored at 5° C. the trimethylamine would have reached this level after about 4 or 5 days or before (Wood, Sigurdsson and Dyer 1942; Dyer and Dyer 1949). Organoleptic examination of the fish showed also that the fish would be judged as spoiled after about this time, 7 days. The

results show that the sodium nitrite concentration is reduced to about 50 p.p.m. before rapid production of trimethylamine occurs.

In a second experiment commercial cod fillets were used which were cut from fish stored longer in ice, although the spoilage had not progressed far enough for accumulation of trimethylamine to occur. They were divided into 3 lots and dipped for about 30 seconds in solutions as follows: (1) Sodium nitrite 0.4%; (2) sodium nitrate 0.4%; (3) sodium nitrite 0.4% plus sodium nitrate 0.4%. Half of each lot of fillets was wrapped in waxed paper, and the remainder was minced and placed in covered beakers. All samples were stored at 5° C. The results of analyses for trimethylamine and nitrite are shown in figs. 2 and 3. The trimethylamine and nitrite values are expressed as mM per
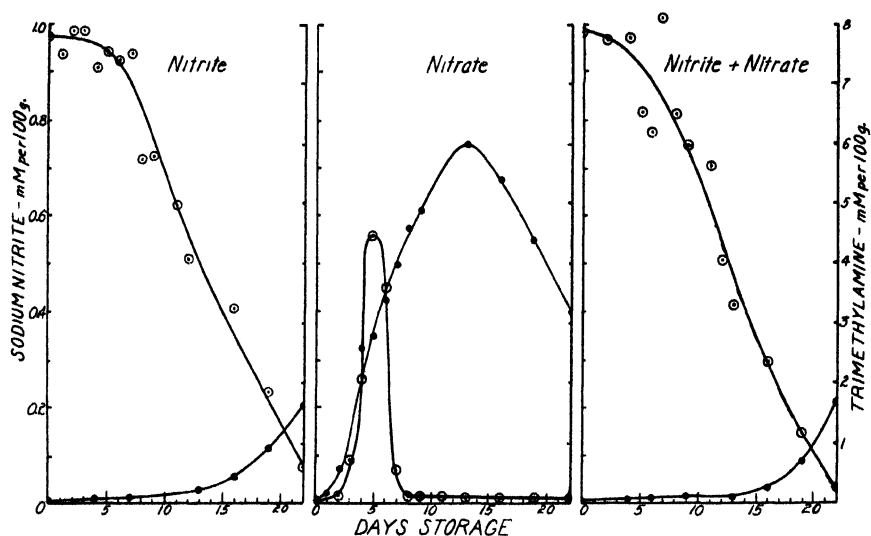
FIGURE 2. Nitrite and trimethylamine oxide reduction in treated fillets stored at 5° C. Nitrite————⊙————; trimethylamine———— ● ————.

100 g. fish. (0.1 mM sodium nitrite per 100 g. is equivalent to 69 p.p.m., and 1.0 mM trimethylamine is equivalent to 14 mg. trimethylamine nitrogen per 100 g.). During the first few days there was a slow reduction of nitrite in the fillets treated with nitrite, followed by a steady decrease from about 5 to 23 days. The slow initial rate of reduction corresponds to the effect of this concentration of nitrite on the growth of bacteria as shown by Castell (1949) in which an incubation period was observed during which little growth occurred and followed by a period of increase in the bacterial count. The pH remained about 6.8 to 7 until about 15 days when a slow increase occurred. The nitrite had been decreased to about 0.7 mM at 22 days. As in fig. 1, the trimethylamine oxide reduction was inhibited until the nitrite had been almost completely reduced. After 22 days the trimethylamine had reached 1 mM nitrogen per 100 g. and was rapidly increasing. The effect of the sodium nitrate will be discussed later.

In the minced samples, fig. 3, the results were the same except that the reduction of the nitrite was faster than in the fillets.

In the third experiment, commercial cod fillets in good state of preservation were used. They were dipped in cold solutions (6° C.) of (1) sodium nitrite, (2) sodium nitrate, and (3) nitrite and nitrate, as in the second experiment, but in this case the initial sodium nitrite concentration, 0.47 mM/100 g., is about half that of the previous experiment. The fillets were stored at 4° C. and analysed as before. Results are shown in fig. 4.
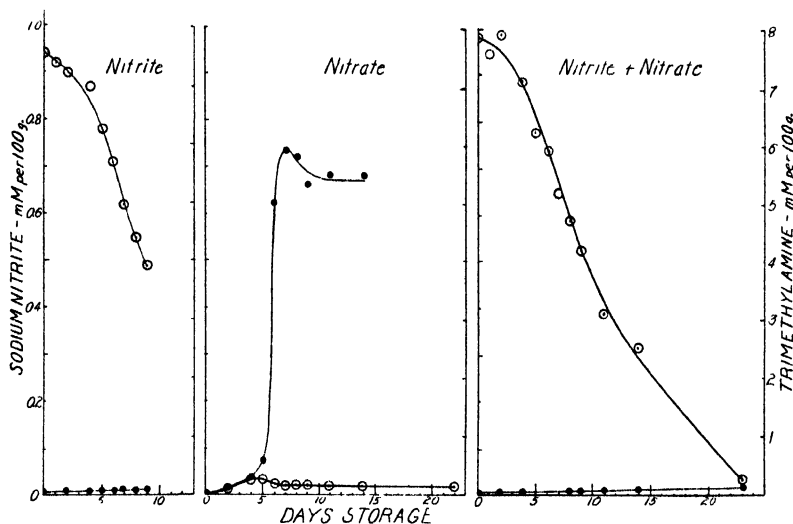
FIGURE 3. Nitrite and trimethylamine oxide reduction in treated and minced fillets stored at 5° C. Nitrite————⊙————; trimethylamine———— ● ————.

A slow reduction in nitrite occurs up to about 11 days when the rate becomes more rapid, the nitrite reaching 0 after 21 days. The initial reduction is slower than in fig. 2, probably because the fillets had a lower bacterial population to start with. The trimethylamine increased very slowly up to about 1.1 mM per 100 g. at 20 days (15 mg. N) at which time the nitrite had decreased to about 0.6 mM per 100 g. and then increased more rapidly. There is some discrepancy, particularly in the results between 12 and 18 days' storage. We believe that these discrepancies are due to errors in sampling, possibly resulting from wide differences in bacterial populations of the fillets analysed. The surface pH (Wood, Sigurdsson and Dyer 1942) slowly increased from 6.6 at the start to 7 at 20 days, and then rapidly increased to 8 and beyond as trimethylamine oxide reduction speeded up.

EFFECT OF SODIUM NITRATE

In fig. 2, it is shown that sodium nitrate in fillets is reduced rapidly to nitrite. Nitrite formation begins after 3 to 4 days, reaching its maximum after 5 days. It is then reduced rapidly, having completely disappeared after 8 days. Nitrate

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had been completely reduced after 6 to 8 days as shown by qualitative tests. In contrast to the effect of nitrite, trimethylamine formation begins between 1 and 2 days' storage, and has reached a value of about 2.5 mM per 100 g. (35 mg. trimethylamine nitrogen) before appreciable nitrite accumulation occurs. Further, the trimethylamine reduction continues to its maximum even in the presence of 0.56 mM of sodium nitrite. The surface pH rises rapidly with the trimethylamine formation reaching 7.5 after 4 days, at which time the nitrite rapidly accumulates. Thus, in this case the nitrite is present only after the pH
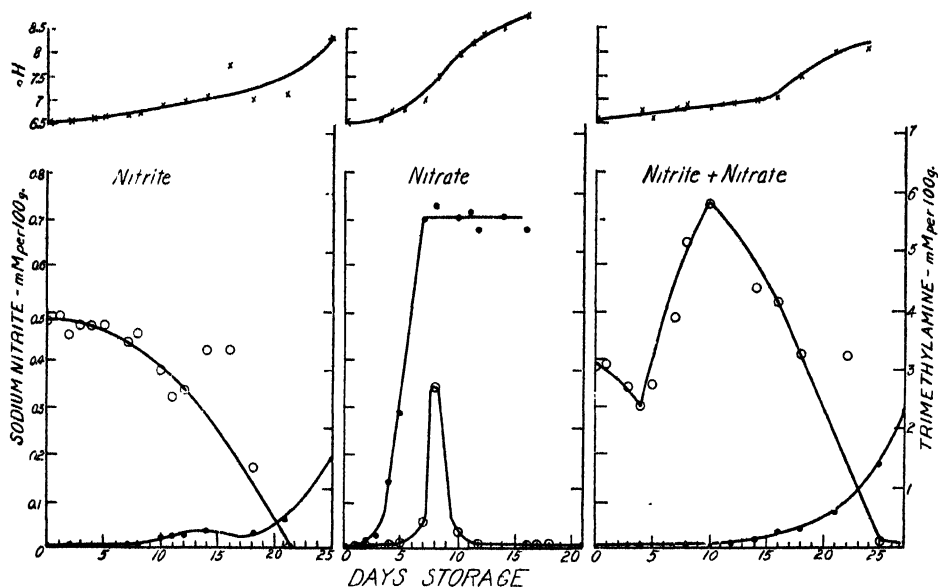
FIGURE 4. Nitrite and nitrate reduction, trimethylamine formation and pH in treated fillets stored at 4° C. Nitrite————⊙————; trimethylamine————●————.

has become alkaline. In the minced fish (fig. 3) there is again no inhibition of trimethylamine production. Here there was no significant accumulation of nitrite, about 0.04 mM per 100 g. being present after 5 days. Since qualitative tests showed that the nitrate rapidly disappeared, the nitrite must have been reduced as fast as it was formed.

In the third experiment (fig. 4) the results are similar to those in fig. 2, except that here the trimethylamine oxide has been completely reduced before nitrite accumulation occurs. Again nitrite formation and its subsequent reduction is very rapid, about 0.35 mM being formed in 1 day and reduced in 1 to 2 days. In this case also the pH has reached 7 to 7.5 while nitrite is accumulating. It should perhaps be emphasized that nitrate reduction to nitrite and the reduction of the latter are taking place simultaneously, and that nitrite accumulates only when it is being formed faster than it is being reduced. Thus, in the nitrate-dipped fillets, the nitrite accumulating is 70 to 85% of that equivalent to the nitrate initially present, if it is assumed that the amount of nitrate

taken up is the same as the nitrite taken up. The indication is, therefore, that the nitrate reduction to nitrite is nearly complete before the nitrite itself is reduced, as might be expected for an enzymic reaction such as this dependent on the oxidation-reduction potential at the site of reduction.

EFFECT OF NITRITE AND NITRATE

In fillets dipped in both sodium nitrate and nitrite, it is shown in fig. 4 that there is an initial reduction of nitrite up to 4 days followed by a rapid accumulation of nitrite from 4 to 9 days, resulting from reduction of nitrate to nitrite. During the formation of this nitrite the pH is acid, about 6.6 to 6.8, in contrast to the fillets treated with nitrate alone. After 9 days the nitrite is rapidly reduced, becoming 0 at about 25 days. This is about 4 days later than the fillets treated with nitrite alone. In this experiment also rapid formation of trimethylamine does not occur until the nitrite level is reduced to about 0.1 mM per 100 g. In fig. 2, the reduction of the nitrate is not nearly so evident, although there is some evidence of a peak after 7 days, followed by a uniform reduction of the nitrite concentration to 0 after about 23 days. In this case and in the minced fish (fig. 3) reduction of nitrate and nitrite seem to occur simultaneously. This is in contrast to the nitrate-treated fish. The difference is that in the latter the pH has increased to above 7 while in the former it is still on the acid side. This agrees with the results of Woods (1938) who found that the nitrate reduction was much faster in alkaline solution than in acid, and the reverse was true for the nitrite reduction.

It will be observed in fig. 2 that there was a loss of trimethylamine after 12 days from the nitrate-treated fish. The pH is 8 or above at this stage so it is not surprising that there should be volatilization of such a volatile base as trimethylamine at this pH. The trimethylamine values for the nitrite-treated fish may thus be slightly low at the final values.

DISCUSSION

Nitrite in fish fillets and in minced fish muscle is rapidly reduced by bacterial enzymes when the fish is stored at temperatures of about 5° C. During this reduction there is considerable bacterial growth at the concentrations of nitrite used (Castell 1949). At the same time there is an inhibition of reduction of trimethylamine oxide to trimethylamine until the nitrite concentration has been reduced to about 50 p.p.m. This parallels the results obtained by Castell, who showed that in fish containing various concentrations of nitrite there was little or no inhibition of trimethylamine formation below 100 p.p.m. sodium nitrite. During this phase the pH of the fish remained between 6.6 and 7. The significance of pH will be discussed fully in a later paper.

It was thought that this inhibition of the amine reduction might be due to a preferential reduction of the nitrite. However, in fig. 4, for example, after 19 days 0.38 mM of nitrite has been reduced in the nitrite-treated samples and at the same time 1.05 mM of trimethylamine per 100 g. fish has been formed.

Since 3 moles hydrogen is required for the enzymic reduction of 1 mole of nitrite and 1 mole hydrogen per mole of trimethylamine oxide, 1.14 mM hydrogen has been used for reduction of the nitrite and 1.05 mM for the trimethylamine oxide. Thus, about the same amount of reduction has taken place of both the nitrite and the oxide and preferential reduction does not seem to occur. It seems more likely that the nitrite has a specific inhibitory action on the trimethylamine oxide reductase, parallel to the effect of nitrite on certain other enzymes (Bernheim 1943, Scarini and Nord 1944, 1945). Again as with these other enzymes, there is some evidence that this effect is greater in acid solution, indicating that the effect is probably due to nitrous acid rather than nitrite. In figs. 2 and 4 it will be seen that when the nitrite is being formed in the nitrate-treated fish, the pH has reached about 7.5 and there seems to be no inhibition of the trimethylamine formation in contrast to the nitrite-treated fish where the pH remains acid. It appears then that the nitrous acid reacts with the trimethylamine oxide reductase, possibly with the formation of diazo compounds with the amino groups as Bernheim (1943) and Sciarini and Nord (1945) have found with carboxylase. Bacterial growth is similarly inhibited by nitrite in acid solution (Meiklejohn 1940; Tarr 1941).

As regards the velocity of reduction of nitrite between 5 and 23 days in fig. 2, about 0.05 mM of nitrite is reduced per day. In the minced fish the rate between 4 and 9 days was about 0.08 mM per day. At a pH of 7.5 to 8 as contrasted with about pH 6.8 above, the reduction is much faster, about 0.39 mM per day after 6 to 7 days (fig. 2). Apparently the reduction in alkaline solution is much faster. However, no account is taken of the difference in bacterial count in the different treatments, which may be considerable. Also, the types of bacteria may be quite different, with similar differences in the activity of the reducing systems. However, this does agree with the fact that nitrite does inhibit growth of bacteria in high concentrations in acid media, and also inhibits other enzymes. The above result is contrary to that of Woods (1938), who found that resting cells of *B. coli* reduce nitrite faster under acid conditions.

Organoleptic examination showed that the fillets usually remained acceptable up to the stage of rapid trimethylamine formation. Thus, the use of nitrite results in an appreciable increase in keeping time, due to the greatly reduced production of objectionable flavours, even though the bacterial growth may not be prevented.

Contrary to the results of Tarr (1944), nitrate in fish fillets is rapidly reduced to nitrite. In fig. 2 about 0.56 mM of nitrite is formed between 3.5 days and 5 days, and in fig. 4 about 0.3 mM between 7 and 8 days. This occurs at a pH of 7 to 7.5. In the presence of nitrite, nitrate is also reduced rapidly. In fig. 4, 0.42 mM of nitrite is formed from 4 days to 9 days, about 0.08 mM per day, compared with about 0.3 mM per day with nitrate alone. The pH is acid, about 6.8, in the presence of the nitrite.

Thus, the nitrite formed from nitrate at a pH acid to the neutral point when nitrite was also present in the fillet should be effective in delaying the reduction of trimethylamine oxide, and indeed this was the case, in contrast to that formed

in the fillets treated with nitrate alone where nitrite is formed under alkaline conditions. It would seem undesirable to treat fillets commercially with a mixture of nitrite and nitrate, since the resulting reduction of nitrate to nitrite would increase the concentration of the latter at certain stages to a level that might produce some toxic symptoms.

In these experiments, trimethylamine formation in the presence of nitrate alone is as fast as, or faster than, is usually found in untreated fillets, although there were no controls. It is concluded that nitrate does not inhibit trimethylamine oxide reduction, nor does nitrite formed *in situ* under these conditions, probably because the medium becomes alkaline when nitrite is formed.

SUMMARY

Bacteria reduce sodium nitrite in cod fillets and minced fish stored at about 5° C. The surface pH remains on the acid side until the nitrite has been reduced.

Rapid reduction of trimethylamine oxide is inhibited until the nitrite concentration has been reduced to about 50 p.p.m., after which trimethylamine is produced rapidly with an accompanying rise in surface pH. This inhibition appears to be due to the action of nitrous acid on the enzymes, reducing the trimethylamine oxide possibly by interaction with the amino groups of the enzyme protein with the formation of a diazo compound.

Organoleptically the fish usually remain acceptable up to the stage of rapid trimethylamine formation. Thus, the use of low concentrations of nitrite results in increased keeping time owing to decreased production of objectionable flavours, even though bacterial growth may not be controlled.

Nitrate is rapidly reduced to nitrite which in turn is itself reduced probably to ammonia or nitrogen. Nitrate does not affect the reduction of trimethylamine oxide, the reduction of the latter being usually complete or nearly so before the nitrate is reduced. Nitrite accumulating by the reduction of nitrate does not inhibit trimethylamine formation. At this stage the pH has reached 7.5 or above in the experiments reported, and it appears that the nitrite inhibits the trimethylamine-oxide-reducing enzyme under acid but not under alkaline conditions, parallel to its effect on bacterial growth and on other enzymes.

Nitrate is reduced in the presence of nitrite, resulting in an increased nitrite concentration. Since the pH remains acid, the increased nitrite results in a longer keeping time.

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INFLUENCE OF THE AMINO ACID - DEXTROSE REACTION ON GROWTH OF LACTIC ACID BACTERIA

By Dyson Rose and Ruth Peterson

INFLUENCE OF THE AMINO ACID – DEXTROSE REACTION ON GROWTH OF LACTIC ACID BACTERIA[1]

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Abstract

Growth of *Lactobacillus arabinosus*, *L. casei*, and *Streptococcus faecalis* (as measured by lactic acid production) was studied in relation to the effects of the products of the amino acid – reducing sugar (Maillard) reaction. Addition of preformed Maillard products to a medium had little or no effect. Medium that had been autoclaved after the addition of dextrose promoted more rapid growth (shorter lag phase) than medium for which the dextrose had been autoclaved separately. This effect could not be traced to the presence of Maillard products, but appeared to be a complex phenomenon depending in part on the Eh of the solution. Destruction of amino nitrogen occurred during autoclaving, and destruction of tryptophan was evident from a comparison of growth response curves. It is concluded that the Maillard reaction affects the growth of these organisms only when an essential amino acid (or other nutrient), present in limiting quantities, is destroyed by the reaction. A serious error may be introduced into microbiological assays for amino acids if the samples to be assayed contain dextrose.

Introduction

Dextrose-containing media for the culture of micro-organisms darken in color when autoclaved. The darkening is a function of time and temperature, and, when it is intense, growth of some organisms is frequently less vigorous. Recent studies have led to recognition of much of the darkening of media during heat sterilization as an example of the amino acid – reducing sugar (Maillard (4)) reaction. It appeared possible, therefore, that products formed by the reaction of amino acids with dextrose might have inhibitory effects on the growth of some species of bacteria, and the present work was undertaken to determine the extent to which this inhibition might affect species commonly used in microbiological assays.

Stanier (11) presented evidence to show that heat sterilization of glucose in the presence of mineral salts rendered it toxic to *Cytophaga*, but Fahraeus (1) was unable to confirm this finding even for the same species. Hill and Patton (3) showed that the growth response of *Streptococcus faecalis* to given trytophan levels was lowered if dextrose was heat sterilized with the other constituents of the medium but concluded (7) that this was due to a destruction of tryptophan.

The studies with *Cytophaga* (1, 11) were conducted using a medium containing no protein or amino acid nitrogen, and which, therefore, could not have contained Maillard products. In studies requiring more complex media, two types of products may be present after heat sterilization (true carameliza-

tion products and the nitrogen-containing products of the Maillard reaction) and it would be desirable to distinguish between them but, to our knowledge, no method is available.

Materials and Methods

Three organisms commonly used for vitamin and amino acid assays were chosen: *Lactobacillus arabinosus* (8014),* *L. casei* (7569),* and *S. faecalis* (9790).* Transfers from stab cultures to a complete liquid medium were made 24 hr. before test solutions were to be inoculated. Inoculum was prepared by centrifuging the organisms from the liquid medium, resuspending them in 2 ml. of 1% saline, and diluting four drops of this suspension with approximately 20 ml. of saline. One drop of dilute suspension was used to inoculate 10 ml. of culture medium.

Two types of media were used in the studies: the synthetic medium used for amino acid assays (similar to that of Stokes *et al.* (12)), and the casein hydrolyzate medium used for niacin or tryptophan assay (similar to that of Greene and Black (2) and Snell and Wright (10)). In some tests, dextrose was added before autoclaving so that Maillard product was formed *in situ*, in others the medium was prepared in the normal manner except that no dextrose was added; then 5 ml. of a 2% solution of dextrose, containing Maillard product if desired, was added to each tube of medium after autoclaving (total volume, 10 ml.). In a few experiments medium containing dextrose was sterilized by Seitz filtration.

Maillard product was formed for subsequent addition to a medium by subjecting a mixture of dextrose and casein hydrolyzate (General Biochemicals Ltd.) to a temperature of 140° F. for several days. In the absence of a suitable method for determining the type of reaction (caramelization or Maillard) that had occurred, all fluorescent compounds formed in the presence of amino acids were assumed to be Maillard products. The concentration of these compounds in the media was estimated by determining the relative fluorescence with a Coleman Model 12 photofluorometer and B_1 and PC_1 filters.

The extent of growth of the organisms was determined by titrating the 10 ml. of culture medium with $0.1 \, N$ sodium hydroxide and the results are presented as milliliters of sodium hydroxide used.

Results

Effect of Various Factors on Initial Growth of the Organisms

The rate of growth of these organisms was compared in medium prepared and autoclaved in the normal manner, medium to which sterile dextrose solution was added after autoclaving, and medium that had been sterilized by Seitz filtration.

* *American Type Culture Collection Numbers.*

The results are given in Figs. 1 and 2, and show that, in contrast to the normally autoclaved medium, little or no growth of *L. arabinosus* or of *L. casei* occurred during the first 24 hr. in medium for which the dextrose was
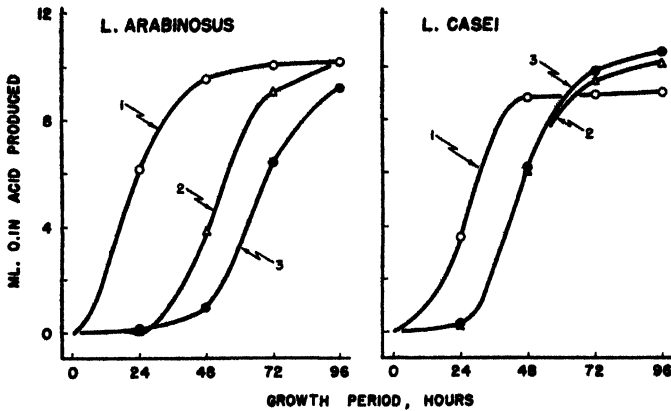
FIG. 1. *Effect of autoclaving amino acid assay medium on acid production.*
1. *Medium autoclaved with dextrose, fluorescence 27.5.*
2. *Medium Seitz filtered, fluorescence 9.0.*
3. *Dextrose autoclaved separately, fluorescence 9.0.*
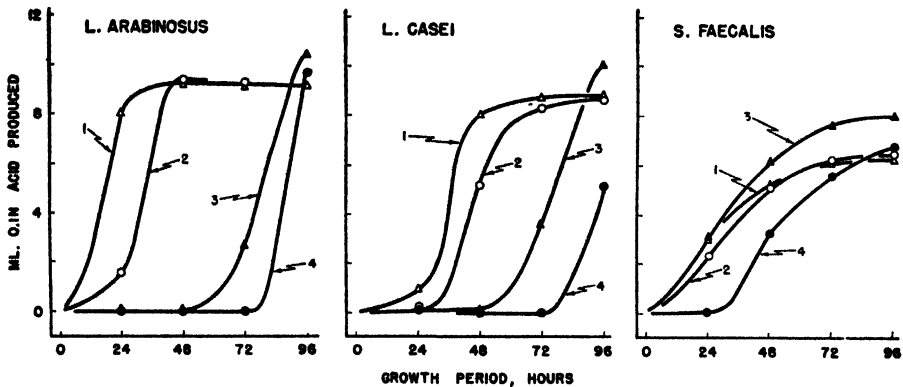
FIG. 2. *Effect of the weight of inoculum on acid production in autoclaved and unautoclaved amino acid assay medium.*
1. *Medium autoclaved with dextrose, fluorescence 64, heavy inoculum.*
2. *Medium autoclaved with dextrose, fluorescence 64, light inoculum.*
3. *Dextrose autoclaved separately, fluorescence 9.0, heavy inoculum.*
4. *Dextrose autoclaved separately, fluorescence 9.0, light inoculum.*

autoclaved separately. In a medium sterilized by Seitz filtration, a similar but less pronounced extension of the lag or induction period was observed. This prolongation of the lag phase was most marked if a light inoculum was used but was evident even in normally inoculated cultures (Fig. 2). *S. faecalis* was much less sensitive to factors inducing the prolonged lag phase than

were the two species of *Lactobacilli;* nevertheless, when a light *S. faecalis* inoculum was used, a slight but distinct prolongation of the lag phase occurred.

Attempts were made to duplicate this stimulatory effect of autoclaving by adding preformed Maillard products. Control tubes were autoclaved after the addition of dextrose; the others received, after autoclaving, a separately sterilized solution of dextrose that contained widely varying amounts of Maillard product. The results were not entirely consistent but indicated that initial growth was not influenced by the mere presence of Maillard product but depended upon some secondary factor introduced when the reaction occurred in the medium.

Results obtained when the initial pH of the medium was carefully controlled are presented in Fig. 3. These data show that the effect of autoclaving on the pH was not a critical factor.
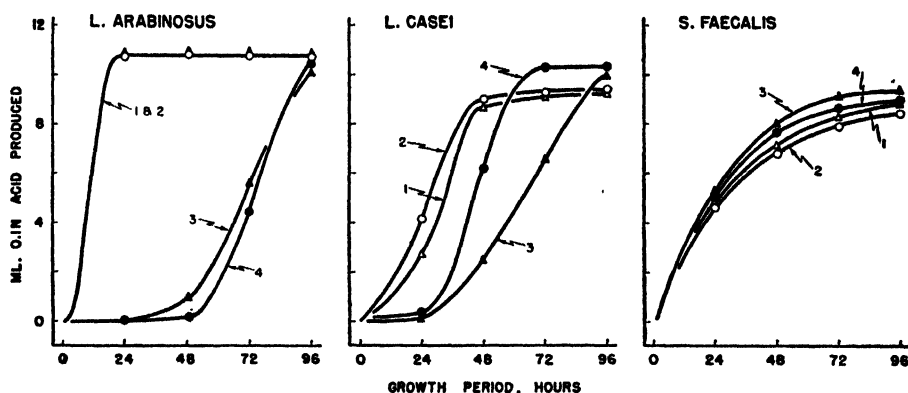
FIG. 3. *Effect of autoclaving amino acid medium, and of pH, on acid production.*
  1. Medium autoclaved with dextrose, initial pH 6.78, final pH 6.51, fluorescence 26.
  2. Medium autoclaved with dextrose, initial pH 6.51, final 6.32, fluorescence 22.
  3. Dextrose autoclaved separately, initial pH 6.78, final 6.78, fluorescence 9.5.
  4. Dextrose autoclaved separately, initial pH 6.51, final 6.52, fluorescence 9.5.

To change the oxidation–reduction potential of the medium a solution of ascorbic acid (20 mgm. in 250 ml.) and dextrose, sterilized by Seitz filtration, was added to one lot of medium before autoclaving. Growth in this lot, as shown in Fig. 4, greatly exceeded that in the tubes for which dextrose had been autoclaved separately and, with two organisms, exceeded that in normally autoclaved medium.

Determination of the *Eh* of these complex solutions gave results of doubtful significance. While the *Eh* of medium containing ascorbic acid was invariably negative (−0.03 to −0.04 v.), that of normal medium varied from + 0.02 to + 0.08 v. Medium autoclaved with dextrose tended to give lower values than that for which the dextrose had been autoclaved separately, but medium with added Maillard product sometimes gave still lower *Eh*

values. Within this range (+ 0.02 to + 0.08 v.) no correlation between the early growth rate of the organisms (24 hr. titer) and the Eh could be found.
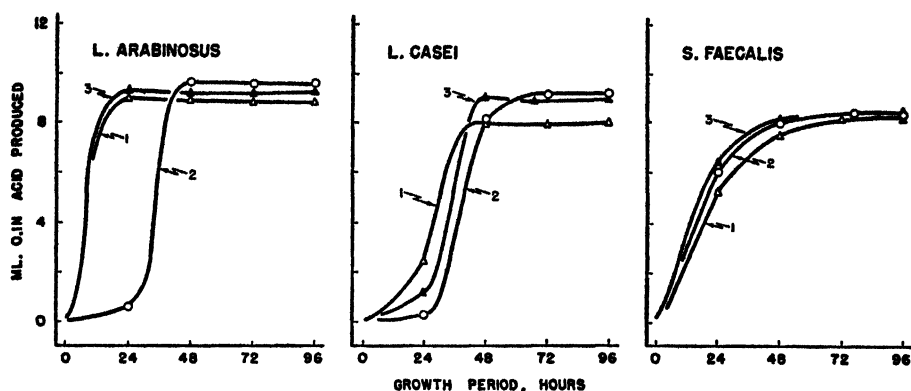
FIG. 4. *Effect of autoclaving tryptophan assay medium, and of ascorbic acid, on acid production.*
1. *Medium autoclaved with dextrose, fluorescence 48.*
2. *Dextrose autoclaved separately, fluorescence 16.5.*
3. *Ascorbic acid added to medium, fluorescence 14.5.*

Effect of Various Factors on Total Growth of the Organisms

When the dextrose is autoclaved with other constituents of the medium, reactions occur that destroy some dextrose. When Maillard products are formed externally to the medium a variable amount of dextrose usually remains unaffected by the reaction and is added to the medium with the Maillard products. This could be avoided by using the insoluble type of Maillard product but such material is not typical of that formed in media under the usual conditions of autoclaving. It was thus impossible to vary the amount of Maillard product in the medium without varying the sugar content but, in spite of this difficulty, attempts were made to determine the effect of Maillard product on total growth of the organisms.

For this purpose, varying amounts of a solution of Maillard products were added to weighed quantities of dextrose and the total volume made to 100 ml. After autoclaving, these solutions were dispensed to tubes of dextrose-free medium. For Expt. I of Table I the amount of dextrose was varied so that the total amount present, if none had been destroyed in the Maillard reaction, would have been 2 gm. per 100 ml. For Expts. II and III, 2 gm. of dextrose was used in each solution and the original dextrose content has been calculated to include that of the dextrose–casein hydrolyzate mixture.

The data presented in Table I show that total acid production by the two *Lactobacilli* paralleled the original dextrose content, and decreased with increasing fluorescence only in Expt. I in which no dextrose was added to offset that destroyed by the Maillard reaction. The total acid production by

TABLE I

EFFECT OF MAILLARD PRODUCT AND DEXTROSE CONCENTRATION
ON THE TOTAL ACID PRODUCTION

Ml. of Maillard solution	Original dextrose, %*	Fluorescence	Ml. of 0.1 N acid produced in 120 hr.		
			L. arabinosus	L. casei	S. faecalis
Expt. I					
0	2.00	7	9.64	10.72	—
2.5	2.00	165	9.75	9.40	—
5.0	2.00	300	8.60	8.20	—
7.5	2.00	415	7.66	7.28	—
10.0	2.00	500	6.43	6.11	—
Expt. II					
0	2.00	11	10.54	10.42	7.59
2.5	2.23	780	11.31	11.27	7.91
5.0	2.45	2250	12.02	12.03	7.98
7.5	2.63	3100	13.07	12.83	8.03
Expt. III					
0	2.00	10	10.44	10.09	8.65
1	2.09	310	10.68	10.30	8.35
2	2.18	560	11.19	10 67	8.31
3	2.27	800	11.26	10.97	8.00

* No. 1 of each experiment contained exactly the dextrose content shown; in the remainder, some dextrose had been destroyed in the formation of Maillard products.

S. faecalis was less influenced by the original dextrose content of the medium and there is some evidence (Expt. III) of a mild toxicity. Even for this species, however, the toxicity is obviously very slight.

To confirm the influence of the available dextrose on total acid production, solutions of dextrose were prepared so that 5 ml. would supply 100, 110, 120, or 130 mgm. per tube. Tubes receiving these amounts were autoclaved after the addition of the dextrose, and other tubes were given exactly 100 mgm. after autoclaving. The results are presented graphically in Fig. 5A and indicate that, with both L. arabinosus and L. casei, acid production from 100 mgm. of dextrose added after autoclaving was equal to that from approximately 107.5 mgm. added before autoclaving. Many of the compounds in the medium, including the Maillard products, interfere in chemical methods of dextrose determination, and the latter are therefore not sufficiently accurate to corroborate this figure.

Similar experiments using S. faecalis did not show a linear relation between total acid production and dextrose content. This is believed to have been due to the low buffering capacity of the medium used.

Loss of an Essential Amino Acid from Media During Autoclaving

Formation of Maillard product in a medium involves the destruction of both dextrose and an amino acid. If the amino acid destroyed is essential, and is present in limiting quantities, its loss will reduce the growth of the

organism.   The adequacy of autoclaved media used in these experiments, and
of a "complete" yeast extract medium, was demonstrated by determining the
growth response to increasing concentrations of dextrose.   Data for *L.*
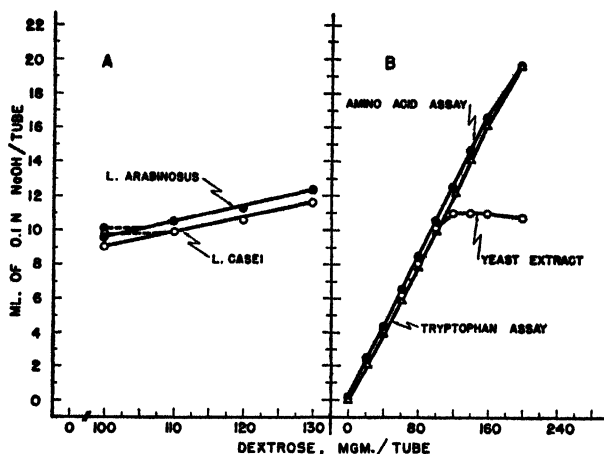
FIG. 5.

A. *Effect of dextrose concentration on the total acid production by L. arabinosus and
L. casei.*

B. *Effect of dextrose concentration on the total acid production by L. arabinosus in three
types of media.*

arabinosus are presented in Fig. 5*B*, and show that a direct linear response is
obtained over a wide range of dextrose concentrations.   With yeast extract
medium some constituent other than dextrose obviously became limiting after
the acid production reached 10.5 ml.   *L. casei* behaved in a similar manner
except that yeast extract medium was approximately equal to the others.

Total amino-nitrogen determinations, made by the micro Van Slyke mano-
metric technique, showed that a considerable destruction of amino acids
occurred during heat sterilization of tryptophan assay medium (Table II)
Higher concentrations of dextrose led to a greater loss of amino nitrogen.

TABLE II

EFFECT OF AUTOCLAVING ON THE AMINO-NITROGEN
CONTENT OF MEDIA

Treatment	Dextrose content, %	Amino-nitrogen, mgm./10 ml.
Seitz filtered	2	3.93
Autoclaved	1	3.79
"	2	3.52
"	3	3.45

A loss of tryptophan was demonstrated by a lowered growth response. Typical curves obtained with *L. arabinosus* (standard microbiological procedures) are presented in Fig. 6A. Proof that the lowered growth response
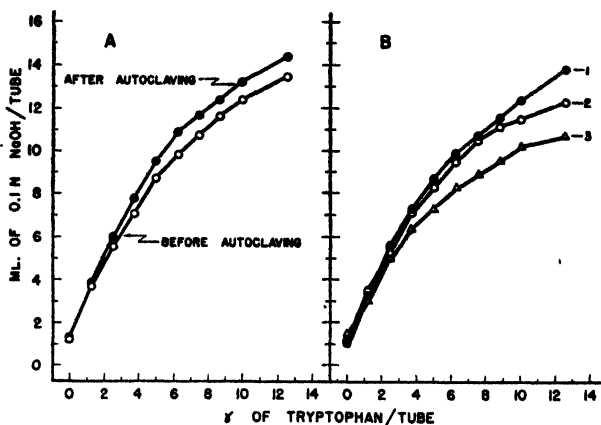
FIG. 6.

A. *Effect of autoclaving on growth response to standard tryptophan levels.*

B. *Effect of dextrose on growth response to standard tryptophan levels.*

    1. *2% dextrose in medium, 0% in tryptophan standard.*

    2. *2% dextrose in medium, 10% in tryptophan standard.*

    3. *4% dextrose in medium, 10% in tryptophan standard.*

was caused by destruction of tryptophan was obtained in a second experiment by adding, after autoclaving, amounts of tryptophan equivalent to the calculated amount destroyed. The resultant curve closely approximated that obtained when all of the tryptophan was added after autoclaving.

The effect of dextrose on the extent of the loss of tryptophan was tested by adding dextrose together with the tryptophan standard so that the amount of dextrose per tube increased progressively with increasing tryptophan. The results, presented graphically in Fig. 6B, show that the addition of dextrose along with the standard tryptophan significantly altered the growth response curve. This effect was more marked when the medium itself had a high dextrose content. It is thus apparent that a serious error may be introduced into microbiological assays for tryptophan if the sample to be assayed contains dextrose, or presumably any other aldehyde, and that the error is greater in media of high initial dextrose levels.

Discussion and Conclusions

Orla-Jensen (6) appears to have been first to recognize the stimulatory effect of autoclaved media on *Lactobacilli*. As a possible explanation he suggested the formation of a complex growth factor. Smiley, Niven, and Sherman (9) observed this phenomenon with *S. salivarius*, and showed that

caramelized dextrose, pyruvic acid, or acetaldehyde, had a similar effect. Niven and Sherman (5) failed to observe any difference in the growth stimulating properties of autoclaved and unautoclaved media when studying *S. faecalis* or *S. zymogenes*, but Rabinowitz and Snell (8) observed poor growth of *S. faecalis* in unautoclaved medium, and found that the addition of a reducing agent, such as ascorbic acid or sodium thioglycollate, overcame the deficiency of the medium.

The results of the present work are essentially in agreement with those quoted. The diverse results previously obtained with *S. faecalis* are, at least in part, explained by the fact that improved growth in autoclaved media was apparent only when a light inoculum was used. In agreement with the results of Rabinowitz and Snell (8) ascorbic acid has been found to stimulate initial growth of the *Lactobacilli*, but it appears probable that this effect was not entirely due to its reducing properties. Maillard products have mild reducing properties, but when formed externally and added to the medium, they failed to stimulate the initial growth. Furthermore, Seitz filtered medium and medium for which the dextrose had been autoclaved separately appeared to possess the same *Eh*, but the former usually supported better initial growth than the latter. Probably some additional factor is involved.

Under the conditions used in our studies, the apparent inhibitions due to moderate concentrations of Maillard product do not appear to have been true inhibitions, but were the result of loss of available nutrient concomitant to the formation of Maillard product. This finding is in agreement with that of Rabinowitz and Snell (8), who showed that, under the conditions used by them, destruction of cystine and cysteine was responsible for the lowered growth. In the present work, destruction of both dextrose and tryptophan was responsible for lowered growth, and probably any essential amino acid, if initially present in limiting quantity, would behave similarly. Thus, there appears to be little direct evidence of toxic effects traceable to Maillard products, especially as regards lactic acid bacteria.

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Fluorescence Development in Egg Powder and in Glucose-Glycine Mixtures

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Fluorescence development in spray-dried egg powder was observed to proceed according to kinetic equations for a normal, first-order reaction with a heat of activation of 28 kg.-cal. and an autocatalytic, first-order reaction with a heat of activation of 5 kg.-cal. Calculations on published data for fluorescence development in dried egg powder showed: heats of activation between 22 and 32 kg.-cal. in the temperature range 20° to 60° C., 8 kg.-cal. from 7° to 24° C., and 2 kg.-cal. from −40° to 4° C. The heat of activation for fluorescence development in mixtures of 2 grams of glucose and 1 gram of glycine, exposed to relative humidities of 75 or 100%, or dissolved in 3 ml. of water, was about 26 kg.-cal., and was reduced by decreasing the relative humidity or by increasing the dilution. Only that phase of fluorescence development which obeyed an equation for a normal, first-order reaction was attributed to an amine-aldehyde reaction.

MEASUREMENTS of fluorescence have been used for many years to detect adulteration or impurities in foodstuffs (*27*) and more recently for assessing deterioration in food products (*2, 8 9, 17, 19, 22, 23, 25, 28, 29, 31, 34*). Loss of eating quality in egg powders is closely related to a fluorescence value recorded in the units of the Coleman photofluorometer scale (*25*). A reaction between aldehydes and amines, first studied by Maillard (*14*), produces fluorescing substances (*13*) which may be one source of off-flavors in dried egg (*1, 3, 4, 16, 20*), although it has not been possible to isolate and purify the fluorescing substances (*3, 18, 21*). According to Enders (*5*), the first step in this reaction is hydration of the aldehyde molecules; the hydrated aldehyde then reacts with the amine group.

Since nuclei often control the rate of reactions in solids (*12*, page 252), fluorescence development in egg powder may be controlled by nuclei of hydrated aldehyde and amine, and hence may obey kinetic equations for a first-order reaction. This paper presents heats of activation for fluorescence increases in stored egg powder, and in mixtures of glucose (to represent an aldehyde) and glycine (to represent an amine), which support this assumption.

KINETICS OF FLUORESCENCE DEVELOPMENT IN EGG POWDER

Previous studies on egg powder showed that increasing the moisture content increased the development of fluorescence (*32*), but measurements on stored biscuits (*15*) indicated that fluorescence development was most rapid when the product contained

12% moisture, and was reduced by increasing or decreasing the amount of moisture.

Since water is important to the reaction, it may be the component necessary to establish an active nucleus from a potential nucleus of amine and aldehyde. If the relative humidity surrounding the reacting mixture is low, the reaction may be controlled by the rate of formation of active nuclei; this rate would depend on rates of sorption and desorption of water molecules which, in turn, would depend on the partial pressure of water vapor. If water is available in great excess, the reaction may be governed by rate-controlling steps similar to those that occur in solution. However, in the presence of intermediate amounts of water every potential nucleus of amine and aldehyde might have water molecules available to it, and would, thereby, be an active nucleus of amine and hydrated aldehyde regardless of the relative humidity. If these active nuclei attained the proper energy level, they would react, and, regardless of the subsequent steps in the reaction, products would be formed according to a first-order, rate-controlling step.

Calculations, based on the data for fluorescence development in plain, whole egg powder stored at 52° and 60° C. (*31*), indicated that fluorescence changes in this product are governed by two different reactions. One had a normal, first-order, rate-controlling step which obeyed the equation:

$$x = 90 (1 - e^{-kt})$$

where x is the fluorescence developed in any time t, 90 approximates the maximum fluorescence value for this type of change, and k is 0.36 at 52° C. and 1.09 at 60°. The other had an autocatalytic, first-order, rate-controlling step which obeyed the equation:
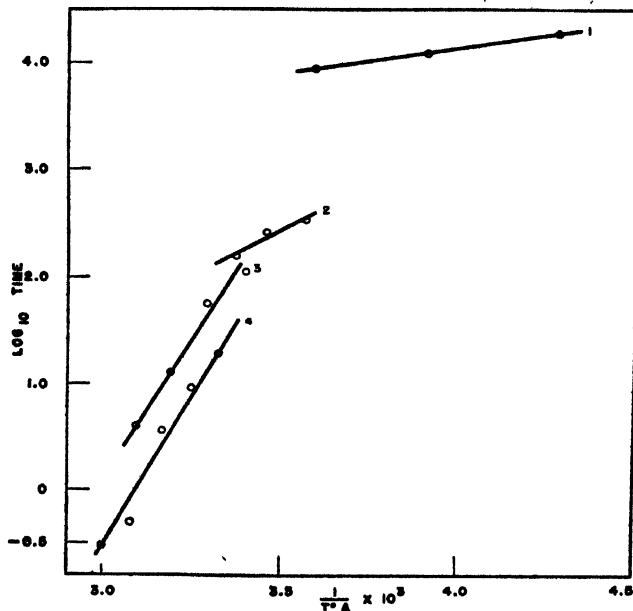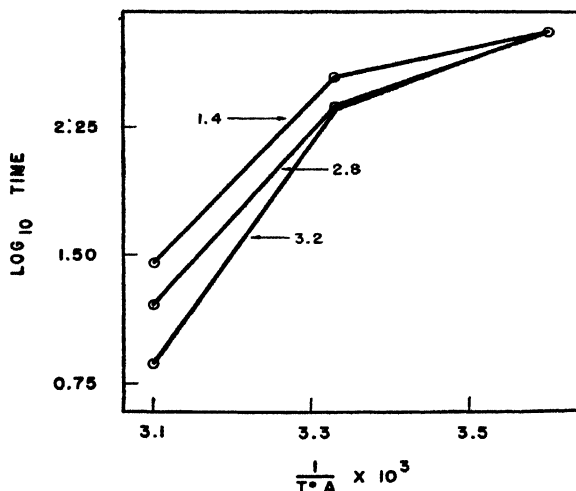

Figure 1. Logarithm of Time (Days) for Plain Egg Powder, Stored at Various Temperatures, to Attain a Fluorescence Value of 50

1. Data from Thistle *et al.* (*30*)
2. Data from White *et al.* (*33*)
3. Data from Stewart *et al.* (*29*)
4. Data for plant II (*31*)

$$\ln \frac{150x}{150 - x} = 150\,kt$$

where x is the fluorescence developed in any time t, 150 approximates the maximum fluoresence value for this type of change, and k is 0.0081 at 52° C. and 0.0100 at 60°. The heats of activation for these reactions were found to be approximately 28 and 5 kg.-cal., respectively. Extrapolation to -40° C. gave rates that, when substituted in these equations, predicted with reasonable accuracy the fluorescence changes observed in egg powder stored at this temperature (*30*).

Other less comprehensive data on fluorescence development in egg powder could not be used to develop similar equations but were used to calculate heats of activation, which confirmed these results. In this subsequent work slight differences in the initial fluorescence values were discounted, and no correction was made for a fluorescence-destroying reaction which is known to occur (apparently first-order with a heat of activation of about 10 kg.-cal.). The error introduced by omitting these factors was less than the variability attributable to differences in the egg powders used in the studies summarized here.

In studies of reaction rates, a predetermined analytical value may be chosen to represent the completion of a definite fraction of a simple kinetic process, and the time to reach this predetermined value is assumed to vary inversely as the rate constant for the process (*12*, page 44). In this work a predetermined fluorescence value was chosen to indicate fluorescence development in a fixed fraction of the nuclei. The time to reach this value might be substituted for t in the equation:

$$\ln t = (Q/RT) - A$$

where $\ln t$ = natural logarithm of time required to reach the predetermined analytical value
T = absolute temperature
R = gas constant, calories
Q = slope of line relating $\ln t$ to $1/RT$ (can be called "heat of activation")

Figure 2. Logarithm of Time (Days) for Sugar-Egg Powder, Stored at Various Temperatures, to Attain a Fluorescence Value of 50

Data from Hay and Pearce (*11*). Moisture contents calculated on the basis of total solids

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Values of A will vary with the predetermined analytical value—e.g., for fluorescence values chosen at intervals of 5 between 25 and 50 (based on data of Stewart *et al.*, *29*), constant A changed according to the equation:

$$A = 38.90 - 0.052\,F$$

where F is the fluorescence value. For this limited range of fluorescence increase, the basic assumptions appeared to be correct, since Q did not change.

Since a fluorescence-destroying reaction occurs in egg powder, and since the reaction between a reducing sugar and an amino acid ultimately results in a charred mass (*14*), the predetermined fluorescence value had to be relatively small to avoid complications from the numerous secondary changes that might occur—namely, 50 for whole egg powder (and 100 for glucose-glycine mixtures described in a later section). The time to attain this value was determined graphically by interpolation or extrapolation or, when necessary, by calculating the equation relating fluorescence value and time.

FLUORESCENCE CHANGES IN SPRAY-DRIED WHOLE EGG

For egg powders stored at temperatures from 20° to 60° C., the logarithm of the time required to reach a fluorescence value of 50, plotted against the reciprocal of the absolute temperature (Figure 1), gave curves with almost the same slopes regardless of the source of the data. At lower temperatures the slopes decreased. Calculations based on these data (Table I) gave heats of activation of 23 to 27 kg.-cal. between 24° and 60° C., of 8 between 7° and 24° C., and of 2 between −40° and 4° C. Figure 2 shows similar behavior in powders prepared to contain two parts of egg solids and one part of sugar solids. This decrease in heat of activation as the temperature decreased indicates a chemical change with two possible reaction paths: The path involving a high heat of activation predominates at high temperatures, and the path with a low heat of activation predominates at low temperatures (*12*, page 45). The values given
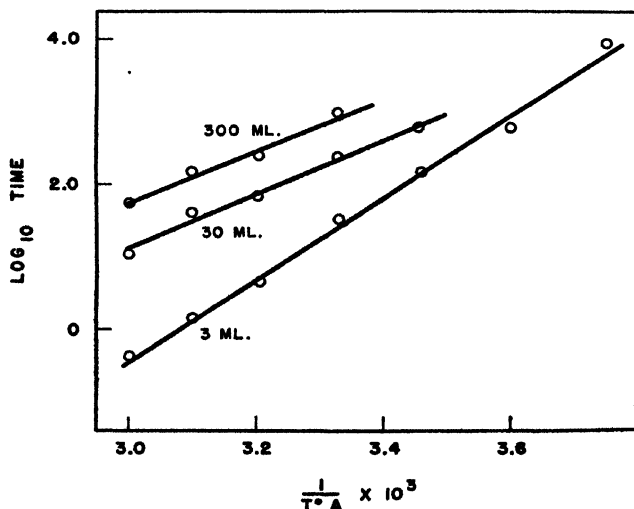
Figure 3. Logarithm of Time (Days) for 2 Grams of Glucose and 1 Gram of Glycine in 3, 30, and 300 Ml. of Water to Attain a Fluorescence Value of 100 When Held at Various Temperatures

here are in close agreement with those reported in the previous section.

As Table II shows, changes in moisture content of plain egg powder from 1.7 to 7% had no effect on the heat of activation.

TABLE I. HEAT OF ACTIVATION, Q, FOR FLUORESCENCE CHANGES IN STORED PLAIN EGG POWDERS

Temp. Range, ° C.	Q, Gram-Cal.	Literature Reference
27 to 60	24,720	(31, plant I)
27 to 60	26,220	(31, plant II)
20 to 50	23,760	(29)
24 to 48	27,130	(26, Fig. 1)
24 to 48	22,020	(26, Fig. 2)
7 to 24	7,620	(33)
−40 to 4	2,160	(30)
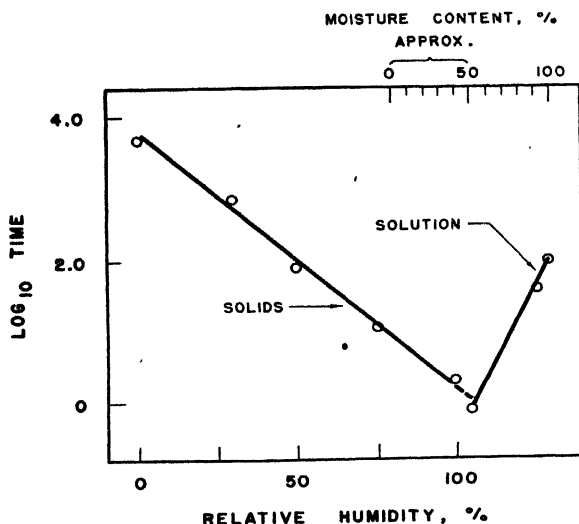
Figure 4. Logarithm of Time (Days) for Solid Glucose-Glycine Mixtures, Exposed to Various Relative Humidities, and for Glucose-Glycine Solutions of Different Concentrations to Attain a Fluorescence Value of 100 When Stored at 49° C.

Trials in this laboratory have indicated that it is impossible to reduce the moisture content of plain egg powder to zero without damaging the powder; therefore, powders free of moisture could not be studied. However, sugar-egg powders dried to a relatively low moisture content have low heats of activation for fluorescence changes (Figure 2 and Table III).

TABLE II. HEAT OF ACTIVATION, Q, AND CHANGE IN CONSTANT A FOR FLUORESCENCE DEVELOPMENT IN PLAIN EGG POWDERS OF DIFFERENT MOISTURE CONTENTS

Temp. Range, ° C.	Moisture Content, %	Q, Gram-Cal.	Change of A with Change of Moisture (M)	Literature Reference
27 to 43	2 to 7	25,990	$38.39 + 0.286\ M$	(32)
27 to 38	1.7 to 4.7	29,500	$43.04 + 0.447\ M$	(24)

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Factor	Temp. Range, °C.	Moisture Content, %	Q, Gram-Cal.
Gas packing (24)			
Air			
Nitrogen	27 to 38	1.7	32,420
Carbon dioxide			
Added substances (26)			
None		2.1	22,020
Sucrose	24 to 48	1.8	14,680
Sodium bicarbonate		2.1	8,070
Added sucrose (10)			
None	27 to 60	4.0	25,490
Sucrose		2.8	24,900

Some other factors that may have affected the heat of activa-
tion for fluorescence development in egg powder are listed in
Table III. Packing in nitrogen or carbon dioxide had no effect,
but the addition of sodium bicarbonate to liquid egg before drying
reduced the heat of activation. If the moisture content was not
too low, the presence of sugar did not alter the value of the heat of
activation although it retarded fluorescence development. This
lends further support to the assumption of active nuclei as the
rate-controlling step; the inert sucrose sugar merely separates
the components that make up these nuclei.
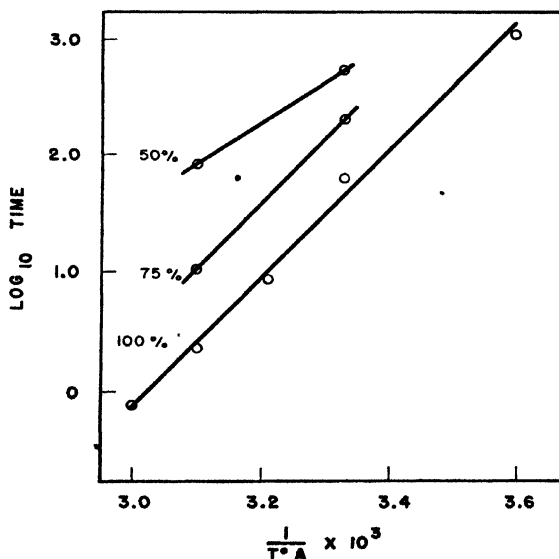
Figure 5. Logarithm of Time (Days) for Solid
Glucose-Glycine Mixtures to Attain a Fluorescence
Value of 100 at Various Temperatures and Humidities

FLUORESCENCE CHANGES IN GLUCOSE AND GLYCINE MIXTURES

Since the foregoing lent support to previous evidence that fluo-
rescing substances were produced in egg powder by two reactions,
it was of interest to relate these observations to fluorescence
development in mixtures of an amine and an aldehyde. This
reaction, in solution, has been studied by many investigators.
Usually the course of the reaction has been examined by following
the loss of amino nitrogen or sugar by a cryoscopic method and by

potentiometric titration (*6, 7*). Results based on these methods were not applicable to the present problem. According to Enders (*5*), products of the reaction are water, carbon dioxide, and a pyrrole-like substance. Previous work indicated similarities between compounds with a pyrrole ring, the fluorescing substances in egg powder, and the products formed by a reaction between reducing sugars and amino acids (*21*). Therefore, the course of the reaction was followed by fluorescence measurements on mixtures stored under conditions which do not produce an increase in fluorescence in the separate components.

MATERIALS AND ANALYSES. Mallinckrodt's anhydrous glucose (analytical reagent grade) and Eastman's ammonia-free glycine were used without further purification. The water was demineralized in a Barnstead unit and had a pH of 5.8.

Fluorescence values were determined by diluting the reacting mixture to 500 ml. with water and measuring the fluorescence of the resulting solution in a Coleman photofluorometer, using the B_1 filter which transmits activating light in the region of 365 millimicrons and the PC-1 filter which transmits fluorescent light above 400 millimicrons (*21*). If the fluorescence exceeded the limits of the photofluorometer scale, further dilutions were made so that all results could be recorded in terms of photofluorometer units. Measurements were made on duplicate samples of reacting mixtures and, under conditions where fluorescence development was slow, at intervals up to one year of storage. For those samples which did not attain the required limit, the time to reach a fluorescence value of 100 was predicted from the changes occurring within the year.

PREPARATION OF REACTING MIXTURES. Fluorescence development was measured in glucose-glycine solutions held at temperatures from $-7°$ to $60°$ C. Mold growth interfered with the study; therefore, concentrated solutions of glucose and glycine were prepared separately and sterilized for 10 minutes at 115° C. Samples of the appropriate dilution were prepared under sterile conditions and stored in sterile flasks. The solutions contained 2 grams of glucose and 1 gram of glycine (approximately equimolar quantities, 0.011 M glucose and 0.013 M glycine) in 3, 30, or 300 ml. of water. Preliminary examination indicated that the pH changes in glucose-glycine solutions were about equal to those occurring in egg powder, when both systems were stored for periods that permitted comparable fluorescence changes. Therefore, it was believed unwise to use buffered solutions since, in addition to retarding pH changes, buffered solutions might introduce undeterminable effects attributable to the various ions present. Furthermore, the use of unbuffered solutions would permit some comparison of changes in solution with changes in the solid state.

For the reaction between solids, 2 grams of glucose and 1 gram of glycine were agitated in a tumble mixer for 30 minutes and then enclosed in a capsule that contained an open vial holding phosphorus pentoxide (approximately 0% relative humidity), saturated salt solutions or water-sulfuric acid mixtures (30, 50, or 75% relative humidity), or water (approximately 100% relative humidity). Fluorescence development in powdered mixtures of glucose and glycine was determined from 4° to 60° C.

Fluorescence development was measured in these powder mixtures spread over areas from 0.32 to 20 sq. cm. Reacting mixtures spread over an area of 0.32 sq.cm. required 150 days to reach a fluorescence value of 100; mixtures spread over areas from 3 to 20 sq. cm. reached this value in 61 days. Since diffusion became the controlling factor in the attainment of moisture equilibrium only for small areas, an area of 10 sq. cm. was chosen for use throughout this work.

RESULTS. Figure 3 and Table IV show that, for solutions at pH 5.8, a tenfold increase in concentration of the reactants—i.e., decreasing the amount of water from 300 to 30 ml.—had no effect on the heat of activation. A hundredfold increase in concentration increased the heat of activation from 16 to 26 kg.-cal.

The reaction in the solid state proceeded at all relative humidities from 0 to 100% (Figure 4), and the logarithm of the time required to reach a fluorescence value of 100 varied inversely with increase in relative humidity. At 50% relative humidity the heat of activation was only 17 kg.-cal.; at 75 and 100%, the

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TABLE IV. HEAT OF ACTIVATION, Q, FOR FLUORESCENCE CHANGES IN MIXTURES CONTAINING 2 GRAMS OF GLUCOSE AND 1 GRAM OF GLYCINE IN CONTACT WITH WATER IN DIFFERENT STATES AND QUANTITIES

State of Water	Quantity of Water	Q, Gram-Cal.
Liquid	300 ml.	15,850
	30 ml.	16,860
	3 ml.	25,840
Vapor (as relative humidity)	100%	24,832
	75%	25,720
	50%	16,850

values were 26 and 25 kg.-cal., respectively (Table IV and Figure 5).

The heats of activation for fluorescence development in these glucose-glycine mixtures did not decrease as the storage temperature was reduced (Figures 3 and 5). Therefore, it seems unlikely that the phase of fluorescence development in egg powder, which obeyed an autocatalytic, first-order equation, can be attributed to the reaction between amines and aldehydes.

DISCUSSION

The heats of activation of about 26 kg.-cal. for fluorescence development in glucose-glycine mixtures, exposed to 75 and 100% relative humidity or in concentrated solution (50% solids content), approximated values of 22 and 32 kg.-cal. for a variety of egg powders of 2 to 7% moisture, and was similar to the value of 28 kg.-cal. calculated for that phase of fluorescence development which obeyed the normal, first-order equation.

However, lower and higher moisture ranges must be considered. When the amount of free water in the glucose-glycine mixture became negligible, the heat of activation for fluorescence development was reduced to about 17 kg.-cal. The value for sugar-egg powder with a moisture content of 1.8% was 15 kg.-cal. If the reaction in the intermediate moisture range is controlled by active nuclei of amine and hydrated aldehyde, this reduction in value indicates that some other factor or factors may control the development of fluorescence at low moisture contents, possibly the rates of sorption and desorption of water molecules.

Fluorescence development at moisture levels higher than 7% has not been studied in dried whole egg powder but has been studied in biscuits. Fluorescence developed most rapidly at about 12% moisture, and increasing the moisture decreased the rate (15). Since Figure 4 shows similar behavior for glucose-glycine mixtures, it is likely that a third set of factors controls fluorescence development at high moisture levels.

These results support previous suggestions that part of the fluorescence development in stored egg powder is attributable to a reaction between amines and aldehydes. In addition, these results indicate that, in the solid state, this reaction originates at nuclei of amine and aldehyde, proceeds by rate-controlling mechanisms that differ with differences in the availability of water, and predominates over other fluorescence producing reactions at temperatures above 24° C.·

ACKNOWLEDGMENT

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LIQUID AND FROZEN EGG

V. VISCOSITY, BAKING QUALITY, AND OTHER MEASUREMENTS ON FROZEN EGG PRODUCTS[1]

By Jesse A. Pearce[2] and C. G. Lavers[3]

Abstract

Freezing irreversibly increased the viscosity of yolk and whole egg, but did not affect the white. Vigorous mechanical treatment before freezing reduced the viscosity of defrosted yolk, white, and whole egg. The viscosity of defrosted yolk and whole egg increased with increase in freezing or thawing time. Mechanical pretreatment or differences in freezing time did not affect the baking quality of defrosted egg products. Freezing reduced the baking quality of yolk and whole egg, but the baking quality improved after storage for about three months at $-10°$ and $0°$ F., and then decreased. A thawing time of four hours resulted in yolk or whole egg of better baking quality than thawing times of 0.03, 24, or 48 hr. There was no relation between viscosity and the baking quality of these egg products. The addition of 2% sodium chloride was equivalent to the addition of 8% sucrose in preserving the foaming quality of frozen yolk.

Introduction

Previous papers in this series have been concerned primarily with the development of tests for measuring eating quality and for determining the solids content of frozen egg. Since frozen egg is used chiefly by the baking trade, it was believed desirable to assess the effects of prefreezing treatment, freezing time, storage time, and thawing time on the baking quality of defrosted egg products. This paper describes the effect of all these factors on baking quality, as assessed by measurements of baking volume or foaming volume (7); and the effect of some of them on other measures, such as viscosity (9), fluorescence value (6), and amino nitrogen (11).

Materials and Methods

Fresh Grade A large eggs were used to prepare the experimental materials, which, unless otherwise stated, were mixed for five minutes in a "Mixmaster" at No. 10 speed, strained through cheese cloth to remove particles of shell and chalaza, poured into Reynold's metal A-10 bags with a capacity of about

one liter (3 by 4 by 5 in.), and frozen in about 10 hr. Freezing was considered complete when a thermocouple in the center of the block of egg reached 20° F. at which point approximately 6% of the water remains unfrozen (8). The time for the liquid to pass from about 45° to 20° F. was recorded as the freezing time.

Defrosting was done in about 15 hr., unless otherwise stated. Defrosting time was defined as the time for the liquid to pass from a temperature of 0° F. to about 35° to 40° F. After defrosting and before testing, the liquid was again mixed at a temperature of 50° F. in a "Mixmaster" for one minute at No. 1 speed. White and whole egg usually became reasonably homogeneous after this mixing period, but as mixing did not make the more viscous samples of yolk homogeneous, their viscosity was tested in the heterogeneous state.

Many of the tests were those used in previous studies in this series (4), and included measurements of pH, reducing sugar, viable bacterial count, volume measurements on test sponge cakes, and foaming volume measurements. Baking and foaming volume measurements were made on defrosted white mixed with freshly separated yolk and on defrosted yolk mixed with freshly separated white. These mixtures were equivalent in composition to freshly broken whole egg For these baking quality tests, the most viscous yolk could be mixed with egg white, but it was more difficult to use than yolk of low viscosity.

Fluorescence was measured on a serum extracted from whole egg (6), and amino nitrogen was determined in whole egg and in yolk by Van Slyke's method (11). Viscosity was measured on defrosted egg liquids at 50° F. with a Gardner–Parks mobilometer (9), which consists of a cylinder to contain the liquid under study, and a piston that is allowed to fall a measured distance through the liquid. The pistonhead can be changed to provide more or less resistance and the weight on the piston can be varied. The 51-hole disk (pistonhead) was used with a total weight of 100 gm including the piston for defrosted white, or whole egg, and with a total weight of 1600 gm. for defrosted yolk. The time in seconds for the piston to fall 20 cm. was taken as a measure of viscosity.

Effect of Prefreezing Treatment on Quality

Mechanical treatment of whole egg before freezing was believed to retard the gelation that occurs during frozen storage (9). This phase of the study was designed to examine the effect of mechanical treatment of whole egg, yolk, and white on viscosity, baking quality, and pH of the stored, frozen product. Strained liquid was treated in four ways: no further treatment, 15 min. additional mixing, dispersion in a laboratory hand homogenizer, and dispersion in a colloid mill. All mixing and dispersing was done in a manner that minimized the introduction of air and the resulting foam. The products were stored at −10° F.

The various mechanical treatments did not affect the baking volume, foaming volume, or pH of whole egg, yolk, or white, whether measured before freezing, after freezing or after frozen storage.

Table I shows that increasing the severity of the mechanical treatment reduced the mobilometer readings, i.e., the viscosity, of unfrozen and defrosted whole egg, yolk, and white. Average readings are given in the table because none of the materials showed any change in viscosity during storage for three

TABLE I

EFFECT OF MECHANICAL TREATMENT ON THE VISCOSITY
OF UNFROZEN AND DEFROSTED EGG PRODUCTS

Treatment	Mobilometer reading, sec.	
	Before freezing	After freezing*
Whole egg		
Mixed		
Mix. 5 min.	3 2	178
Mix 20 min.	2.4	151
Dispersed		
Mix 5 min. + homogenizer	1.5	10
Mix 5 min. + colloid mill	1.3	10
Yolk (1600 gm. weight on piston when used on defrosted yolk)		
Mixed		
Mix 5 min.	27	455
Mix 20 min.	26	315
Dispersed		
Mix 5 min. + homogenizer	19	236
Mix 5 min. + colloid mill	18	129
White		
Mixed		
Mix 5 min.	6 5	4 0
Mix 20 min.	2.9	2 6
Dispersed		
Mix 5 min. + homogenizer	1 3	1.3
Mix 5 min. + colloid mill	1.1	1.1

*Average after storage for 0, 1, 2, and 3 months at − 10° F.

months at −10° F. Freezing did not change the viscosity of egg white, regardless of the treatment. Freezing increased the viscosity of the mixed (Mixmaster only) whole egg about 60 times but only increased the viscosity of the dispersed (colloid mill or homogenizer) whole egg by about eight times. Freezing caused so great an increase in the viscosity of yolk that the weight on the piston had to be increased from 100 to 1600 gm., which interferes with comparisons of the data. Nevertheless there is evidence that dispersed yolk changes less in viscosity during freezing than the mixed yolk.

Effect of Freezing Time on Quality

The liquid was frozen at different temperatures and with different thicknesses of insulation around the Reynold's metal containers, to provide different freezing rates. The bacterial count in the initial, laboratory-prepared material was only about 50 per ml. A count of the same order was observed in products frozen in less than 12 hr., but the count increased to 300 per ml. in product frozen in about 24 hr. and to 1000 per ml. in product frozen in 100 hr. High bacterial counts are usually observed in the cores of commercially prepared cans of egg that have been frozen too slowly. However, after a period of frozen storage, there is usually a marked decrease in the bacterial count (2, 12).
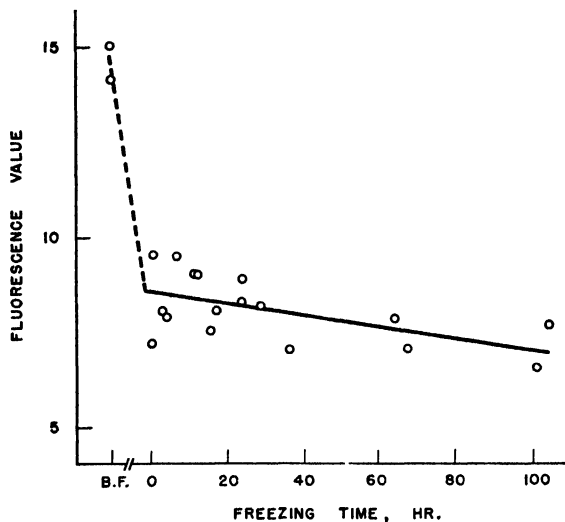
FIG. 1.   *Effect of time of freezing on the fluorescence value of a serum extracted from whole egg.*

A visual examination of the effect of freezing time on color and structure showed that material frozen in 10 min. was light yellow in color. As the freezing time was increased, the color of the product deepened, and at a freezing time of 110 hr. became a dark orange. The frozen product had a laminar structure. If frozen in 10 min., the flakes were alternately opaque and transparent and about 0.001 in. thick. If frozen in 25 hr. the flakes were about 0.05 in. thick, while material frozen in 60 and 100 hr. had a granular structure to a depth of about 2 in., but the center had a laminar structure in which the transparent flakes were about 0.1 in. thick.

Freezing and the time of freezing had no effect on either the reducing sugar or the amino nitrogen content of eggs. The reducing sugar content of whole egg remained at 0.38 mgm. per 100 ml. of egg, regardless of the freezing time. The amino nitrogen content of 5-ml. samples of whole egg and of yolk was 6.90 and 9.66 mgm. respectively, whether frozen or unfrozen.

Fig. 1 shows that freezing appreciably reduced the fluorescence of a serum extracted from whole egg. Increasing the freezing time from about 10 min. to 100 hr. reduced the fluorescence of this extract still further. It is unlikely that this is due to factors other than the rate of freezing, since all measurements shown were made at the same time. These results differ from some other published information. The fluorescence of dried eggs, prepared from liquid egg frozen at different rates, increased with increase in freezing time (5); but freezing and defrosting of extracts containing these fluorescing substances caused a reduction in fluorescence (3). Prolonged storage of frozen egg caused an increase in fluorescing substances (4).
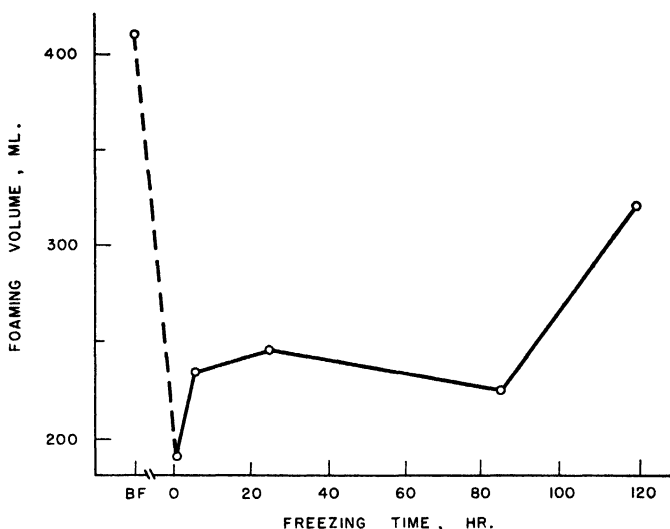
FIG. 2. *Effect of freezing time of yolk on the foaming volume of defrosted yolk mixed with unfrozen white.*

In general, freezing time had little effect on the foaming volume of defrosted whole egg or egg white, but it did affect foaming in defrosted yolk. Unfrozen whole egg had a foaming volume of about 400 ml. Frozen whole egg had a foaming volume of about 375 ml., regardless of the freezing time, while the foaming volume for mixtures of frozen white and unfrozen yolk was about 390 ml. regardless of the freezing time of the white. Fig. 2 shows that freezing yolk in about 100 hr. gave a product which, when mixed with an amount of unfrozen white to give whole egg liquid of correct proportions, had a foaming volume of about 300 ml., while yolk frozen in 10 min. had a foaming volume of only about 200 ml.

The Effect of Added Salt and Sugar on Frozen Yolk

Some explanations have been given for the effectiveness of sodium chloride and sucrose in reducing gelation in frozen yolk (1, 9). Since these substances are used commercially for this purpose (10), it was of interest to see if their

use also affected baking quality of yolk as reflected by foaming volume measurements. Therefore, 2, 4, and 8% of these compounds were added to yolk before freezing, and the samples were examined immediately after defrosting.

Fig. 3 shows that 2% sodium chloride had a marked effect in retaining the foaming volume of frozen yolk, and that increasing the amount of salt had
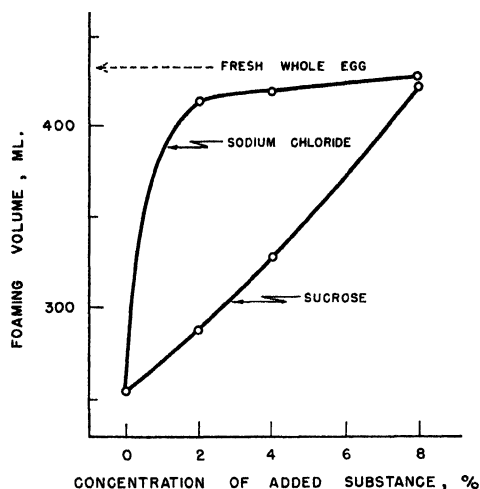

FIG. 3. *Effect of added sodium chloride and sucrose on the foaming volume of defrosted yolk and unfrozen white.*

only a slight additional effect. Increasing the amount of sucrose up to 8% had a progressively greater effect in retaining foaming volume: 8% sucrose gave a product that was about equal to fresh egg.

Effect of Storage Time on Quality

An earlier study in these laboratories indicated a decrease in the baking quality of freshly frozen whole egg (4). Therefore, it was of interest to re-
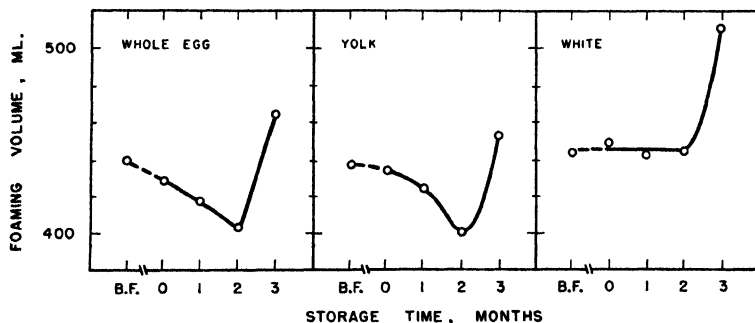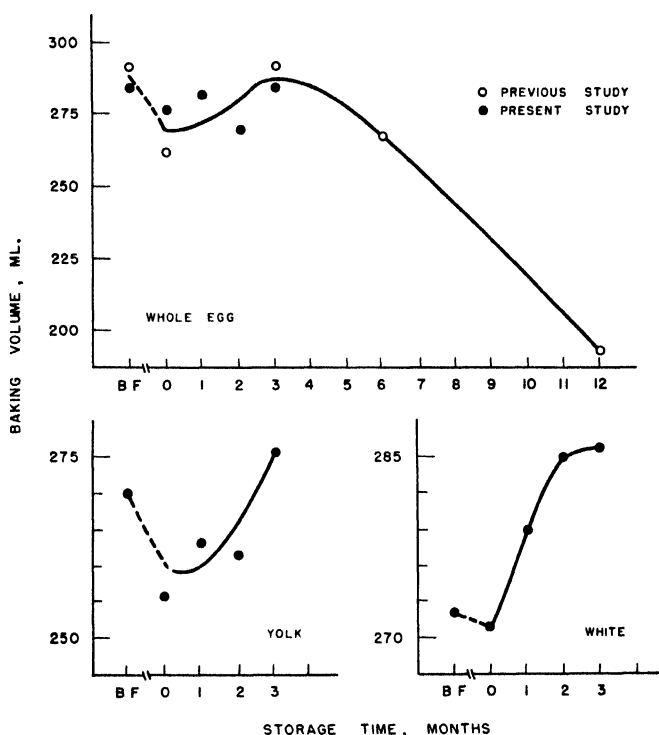

FIG. 4. *Changes in the foaming volume of stored frozen egg products: defrosted yolk mixed with unfrozen white, and defrosted white mixed with unfrozen yolk.*

examine the effect of storage up to about three months at $-10°$ F. on the quality of yolk, white, and whole egg.

Freezing and time of frozen storage effected measurable pH changes, in white and in whole egg, but pH could not be measured in defrosted yolk because of its high viscosity. Egg white had a pH of 9.0 before freezing, which increased to 9.1 after freezing and then gradually decreased to 8.9 at the end of three months' storage. Whole egg had a pH of 7.5 before freezing, which became 7.6 after freezing and then gradually increased to 7.8 after three months' storage. These pH changes in frozen whole egg were similar to those observed previously (4).

Fig. 4 shows that both whole egg and yolk decreased in foaming volume during freezing and the first two months of frozen storage, and then increased

FIG 5 *Changes in the baking volume of sponge cakes made from frozen egg products defrosted yolk mixed with unfrozen white, and defrosted white mixed with unfrozen yolk*

in foaming volume   The foaming volume of egg white remained constant during the first two months of storage, and increased during the third month. Earlier work showed that 12 months' frozen storage appreciably reduced the foaming volume of frozen whole egg (4).

Fig. 5 shows the reduction in baking quality resulting from freezing yolk or whole egg and the subsequent increase in quality after about three months'

storage. The baking quality of egg white appeared to be unchanged by freezing but to increase during the first three months' storage. In general, foaming volume and baking volume show similar changes during frozen storage and the results presented here support previous observations on stored, frozen whole egg (4).

Effect of Freezing and Thawing Time on Quality

During the preceding phase of this study, there was some evidence that thawing time might also have some effect on quality. Therefore, a more comprehensive study was made to examine the effects of freezing time (0.2, 4, 16, and 39 hr.), thawing time (0.03, 4, 24, and 48 hr.), and storage time (0, 1, 2, 4, and 8 months) at 0° F. on viscosity and baking volume of yolk and whole egg.

Just as this study commenced, the oven used in previous baking studies broke down. With the temporary equipment used, pending receipt of a new oven, the cakes were about 30 ml. smaller than usual. Therefore, while the results for this portion of the work are comparable within themselves, they cannot be readily compared with those of previous work.

The changes in viscosity and baking volume were assessed by an analysis of variance. Each of the factors under study had a significant effect on the viscosity of defrosted yolk and whole egg, but only thawing time and storage time had a significant effect on baking quality.

Fig. 6 shows the similarity in effect of these factors on the viscosity of both yolk and whole egg, except that yolk was much more viscous than whole egg. In general, increases in freezing time from 0.2 to 39 hr. caused progressive increases in viscosity; increases in defrosting time from 0.03 to 24 hr. caused increases in viscosity, but increases in thawing time from 24 to 48 hr. caused no further increase in viscosity. Storage time caused no increase in viscosity if the product was frozen or defrosted in four hours or less, but increasing the storage time increased the viscosity in product which had been frozen or defrosted in longer times. Measurements elsewhere had indicated maximum viscosity in defrosted whole egg after two to four months' storage (9); the present results showed a progressive increase in viscosity of slowly frozen or defrosted egg throughout an eight month storage period at 0° F.

In general, while viscosity was not related to the baking volume of defrosted yolk or whole egg, material of low viscosity was more easily prepared for baking. Frozen yolk was much more viscous than frozen whole egg, and usually gave cakes about 20 ml. smaller. However, the difference in cake volume was attributable to factors other than the freezing operation, since cakes made from yolks that had been mechanically mixed, but not frozen, and added back to white also gave cakes about 20 ml. smaller than those from whole egg (Fig. 5).
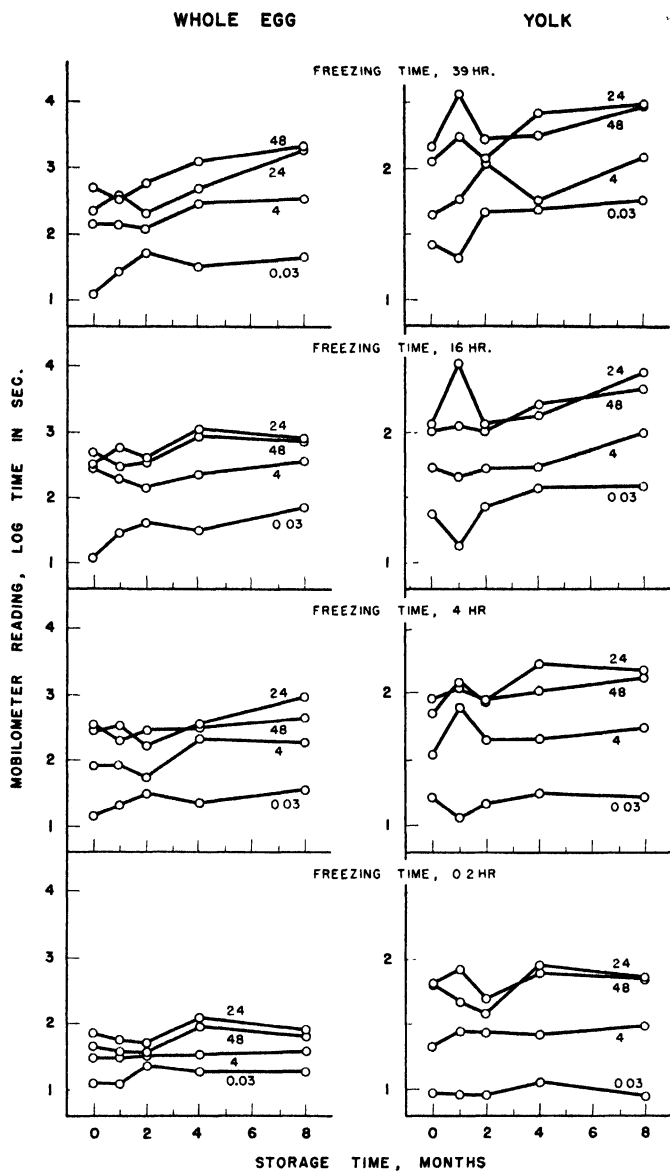
WHOLE EGG   YOLK

FIG. 6.   *Effect of freezing time, thawing time (small labelled figures give the thawing time in hours), and storage time on the viscosity of defrosted yolk and whole egg.*

Fig. 7 shows that product thawed in four hours gave cakes with greater volume than product thawed in shorter or longer thawing times.   While these differences were statistically significant, the increase in baking volume was only about 3%, which may not be of practical value in commercial processing.

Storage time had effects on the baking volume of cakes made from defrosted whole egg similar to those shown in Fig. 5.   The baking volume of defrosted yolk was decreased by freezing and increased after four months' storage in a manner similar to that shown in Fig. 4; but in addition, there was evidence that the baking volume of defrosted yolk decreased between four and eight months' storage.
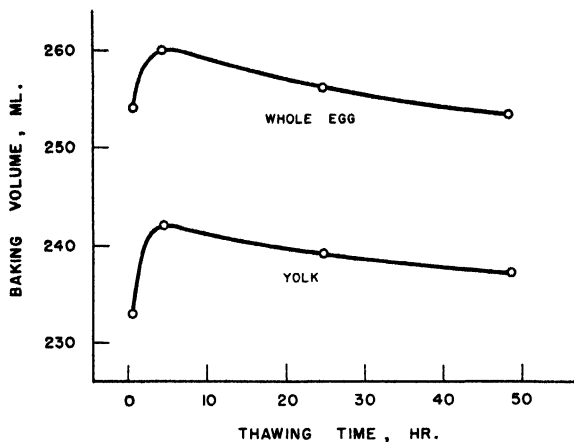
FIG. 7.   *Effect of thawing time on the baking volume of sponge cakes made from defrosted whole egg, and from defrosted yolk mixed with unfrozen white.   Values averaged over all freezing times and storage times.*

Acknowledgments

The authors wish to express their thanks to Miss S. Jegard, Biochemist, Food Investigations, who did the baking tests; and to Mr. J. G. V. Taylor, Laboratory Assistant, Food Investigations, who was responsible for the viscosity measurements and the statistical computations.

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FROZEN STORAGE OF POULTRY

V. EFFECTS OF SOME PROCESSING FACTORS ON QUALITY[1]

By Jesse A. Pearce[2] and C. G. Lavers[3]

Abstract

Examination of a number of phases of poultry processing showed that birds of optimum quality were obtained by: using a 90 sec. bleeding time; dipping in a semi-scald tank for 40 sec. at 128° F.; operating both shafts of the rough-plucker at the same speeds; operating the rough-plucker at conditions such that the rubber fingers did not exert a force greater than 11 lb. on the carcass; and carefully training operators of hand-roughers. Splitting the carcasses up the back, eviscerating, and packing flat reduced the space required for packing to less than 50% of that required by present methods, and still permitted reassembly of the bird into a presentable form for roasting. An impact dynamo-meter was useful in detecting unfrozen spots in the birds. Injecting various solutions into the carcass before freezing, and measuring the drip from the defrosted muscle, showed that maintaining the proper ratio of salts and water in the muscle reduced drip. Bleeding time had no effect on development of off-odors in stored poultry but evisceration before storage markedly reduced development of off-odor on the eviscerated surface and in the thigh meat. Coating eviscerated birds before freezing with carrageenin gel containing 6% sodium chloride delayed development of off-odor on eviscerated surfaces of defrosted carcasses.

Introduction

Earlier papers in this series described the effects of precooling, rates of freezing, and frozen storage on the quality of poultry. However, little published information is available with respect to effects of killing and plucking procedures on quality of frozen poultry. The present paper describes studies on effects of different intervals between killing and entry into the semi-scald tank on dressing out and keeping qualities; detection of unfrozen cores in carcasses; methods that reduce the space required for packing and storage; and preservation of the eviscerated surface after defrosting.

Methods

Many of the appraisals were subjective, and, since they dealt with factors affecting the grading, were made by inspectors of the Dominion Department of Agriculture according to Canadian regulations (2). Other subjective appraisals were made by taste panels recruited from the staff of the National Research Laboratories.

The various quantitative methods are described in those sections in which they were used.

The procedure used in dressing poultry in many Canadian processing plants and used in this study is briefly as follows. The birds are killed, allowed to bleed for a short period (bleeding time) passed through a tank of violently agitated warm water (semi-scald tank), and then between the rotating shafts of an automatic rough-plucker where hollow rubber fingers beat off most of the feathers. The fingers, about 8½ in. long and 5/8 in. in diameter, are placed in rows on the shafts, which are about 5½ in. in diameter. The feathers remaining after rough-plucking are removed by a hand-rougher or by hand. The hand-rougher usually consists of a revolving drum, about 22 in. in diameter, with rubber fingers about 4 in. long on its surface. The shape of these fingers varies— some are circular and hollow, others are square and solid. The birds are held against these fingers by hand and the remainder of the coarse feathers are removed. After the birds leave the hand-rougher, pin-feathers are removed by hand.

Some plants have additional equipment for eviscerating birds, but in these studies, evisceration was done in the laboratory by hand.

Prefreezing Processing

Effect of Bleeding Time on Quality

In this phase of the study, the intervals allowed between killing and entry of the carcass into the semi-scald tank were 30 and 90 sec., since the use of longer or shorter times or of more than two time intervals caused too much

TABLE I

EFFECT OF 30-SEC. AND 90-SEC. INTERVALS BETWEEN STICKING AND SEMI-SCALDING ON PROCESSING DEFECTS IN POULTRY

Defect	Defective birds, %	
	30-sec.	90-sec.
Chicken		
Poor feather removal	12 8	1 5
Scalding	55 5*	45 0*
Rubbing	52 0	39.7
Skin bleeding	43 3	15 2
Fowl		
·Poor feather removal	9.2*	8.2*
Scalding	31.7*	29 4*
Rubbing	33 6	18 2
Skin bleeding	7.3	1 2

No significant difference between the values for the 30-sec. and 90-sec. intervals.

inconvenience in the commercial plant.   Unfortunately, the automatic control on the semi-scald tank was not operating on the day of this trial, and the temperature in the tank had to be controlled by hand.   This resulted in temperature variation in the water from 128° to 132° F.   The birds remained in the tank for 45 sec.   One shaft of the rough-plucker was turning at 330 r.p.m. and the other at 290 r.p.m.   The hand-rougher was operated at 360 r.p.m.

In all, about 800 birds were processed, about 400 at each bleeding time. The results (Table I) showed that birds classed as chicken suffered more defects than birds classed as fowl.   For chicken, the short bleeding time reduced the efficacy of feather removal by the rough-plucker.   On the other hand, feather removal was impossible if bleeding time was prolonged for five minutes.   Rubbing, i.e., abrasion of the skin by the rubber fingers, was greater in birds bled for 30 sec. and is believed to be a direct reflection of the increased handling required to remove feathers on the hand-rougher.   A 90 sec. bleeding time effectively reduced the incidence of skin bleeding.   In general, these results show that a 90 sec. bleeding time is more desirable than a 30 sec. bleeding time; and for chicken in particular, a 90 sec. bleeding time effected a marked reduction in the number of feathers that must be removed by the hand-rougher.

TABLE II

EFFECT OF TEMPERATURE AND TIME IN SEMI-SCALD TANK ON FORCE REQUIRED TO REMOVE FEATHERS FROM POULTRY

(10 to 40 birds processed at each condition)

Temp., ° F.	Time, sec.	Force, oz.		
		Plant A	Plant B	
		Chicken	Fowl	Chicken
125	30	15	22	24
	40	13		15
	50			15
128	20	12		
	30	14	20	23
	40	15		11
130	20			9
	30			11
132	15	13		10
	20	16		
	30	8		
	40	7		
134	15	15		
	20	8		
136	15	5		

Effect of Time and Temperature in the Semi-scald Tank

The time that birds are in the semi-scald tank and the temperature of the water affects not only the quality of the dressed birds but also the effectiveness of the plucking operations.

Table II shows the effect of conditions in the semi-scald tank on the average force required to remove small feathers from the wings and tail (feathers clamped to spring scale). Work was done at Plant A early in September, when birds were still moulting. Relatively little force was required to remove the feathers, and excessive times and temperatures were necessary to effect any reduction in force required. Work was done in Plant B in late November, and, at the lower temperatures and shorter dipping times, the force required to remove the feathers was generally much higher than for September birds. The effect of time of year on the ease of feather removal requires more study. However, for the two periods studied here, approximately uniform resistance might be obtained by the use of appropriate dipping times and temperatures. In general, these results show that the force required to remove feathers from the birds is reduced by increasing the water temperature in the semi-scald tank and by increasing the time of dipping.

Table III shows that the effect of semi-scald tank operation on damage (combined scalding and rubbing) to the carcass is complicated by plucking operations. In Plant A, the birds were examined after passage through a rough-plucker with shafts rotating at 380 r.p.m. and a hand-rougher operating at 130 r.p.m. In Plant B, the birds were examined after passage through a rough-plucker only, with shafts turning at 360 r.p.m. Birds processed in Plant A at the intermediate temperatures showed least damage at the longest dipping times, while birds processed in Plant B showed greatest damage at the longest dipping times. This discrepancy was apparently due to the use of the hand-rougher in Plant A. At the longer dipping times, feathers were more easily removed by the hand-rougher, which reduced the damage from rubbing. From these results and the results in Table II, dipping for 40 sec. in a tank containing water at 128° F. seemed most desirable, if the operation of the two types of pluckers was satisfactory.

Effect of Plucker Operation on Quality

This study was made in only one plant. To eliminate damage from scalding, the water in the semi-scald tank was at 125° F. and the dipping time 30 sec. This temperature and time combination is lower than that recommended in the previous section and feather removal in the rough-plucker was less complete.

Table IV shows that the amount by which the rubber fingers overlap, from a gap of 2½ in. to an overlap of 3½ in., had little effect on rough-plucker operation but that the two shafts had to be operated at the same speed if excessive rubbing was to be avoided. If the shafts were operating at the same speed, the least rubbing damage occurred when the rubber fingers did

TABLE III

EFFECT OF TEMPERATURE AND TIME IN SEMI-SCALD TANK ON FEATHER REMOVAL
AND DAMAGE

(10 to 40 birds examined at each condition)

Temp., ° F.	Time, sec.	Plant A	Plant B	
		Birds damaged, %	Feather removal	Birds damaged, %
125	30	10	Fair	10
	40	50	Good	58
128	20			
	30	60	Good	50
	40	25	Good	80
130	20		Good	66
	30		Good	90
132	15	100	Fair to Good	81
	20	80		
	30	88		
	40	66		
134	15	55		
	20	90		
136	15	100		

TABLE IV

FACTORS IN ROUGH-PLUCKER OPERATION AFFECTING FORCE ON THE BIRD, FEATHER REMOVAL,
AND DAMAGE

(10 to 40 birds examined at each operating condition)

Rough plucker			Maximum downward force on birds, lb.	Feather removal	Damaged birds, %
Speed, r.p.m.	Overlap of fingers, in.				
	Entrance	Exit			
Shafts at same speed					
270	$2\frac{1}{2}$	0	7 0	Poor	0
	$3\frac{1}{2}$	$1\frac{1}{2}$	8 0	Poor	0
360	0	0	8 8	Fair	20
	1	1	10.6	Fair	10
440	$2\frac{1}{2}$	0	13 6	Good	85
Shafts at different speeds					
330, 340	1	0	9 8	Fair	80
	$3\frac{1}{2}$	$1\frac{1}{2}$	7 4	Fair	77
380, 410	0	$-2\frac{1}{2}$	11 2	Good	75
	$2\frac{1}{2}$	0	13.3	Good	77

not exert a maximum force (measured by a spring scale) of more than about 11 lb. on the bird's carcass.

Table V shows that use of the hand-rougher (335 r.p.m.) under semi-scalding conditions approximating those described above doubled the damage to both chicken and fowl. Since little mechanical change can be made in this hand-rougher, the operators must be given special training in its use to avoid excessive damage at this stage of the operation.

TABLE V

EFFECT OF HAND PLUCKER ON DAMAGE TO BIRDS

(30 birds of each type examined at each operating condition)

Equipment used	Birds damaged, %	
	Chicken	Fowl
Rough plucker only	36	42
Rough and hand plucker	62	83

Packing Poultry for Freezing

Current methods of packing poultry waste space, so it was of interest to examine other methods. For this purpose, New York dressed birds weighing 60 lb. per doz. were used, which, by current packing methods, require a box with inside dimensions of 24 by $14\frac{1}{2}$ by $7\frac{1}{2}$ in., i.e., approximately 2600 cu. in. (Fig. 1). Eviscerating the birds and removing the heads and feet reduced the size of the box required to 24 by $14\frac{1}{2}$ by $5\frac{1}{2}$ in., i.e., to approximately 1900 cu. in.

Further comparisons were made using eviscerated birds. Various methods, based on cutting the bird through the center of the ventral cavity or on cutting the bird completely in half, were tried and discarded, since the bird could not be reassembled to make presentable roast chicken and since the breast muscles were exposed with the added danger of spoilage in the most meaty portion of the bird. Splitting birds up the back reduced the time required for evisceration and permitted easy reassembly for roasting, but packing with the back halves of two birds in the body cavity of the third saved little space.

Cutting the birds up the back and flattening by cracking the ribs provided packing in a minimum of space and still permitted reassembly into a presentable form for roasting. Two methods of packing were selected from the large number possible. In the first, the flattened birds were packed on edge with the neck or thighs alternately toward the bottom of the box. This pack required a box with internal dimensions of 21 by 8 by $9\frac{1}{2}$ in., i.e., approximately 1600 cu. in. In the second (Fig. 2), the birds were packed in three columns with the breast surface of one bird in the eviscerated cavity
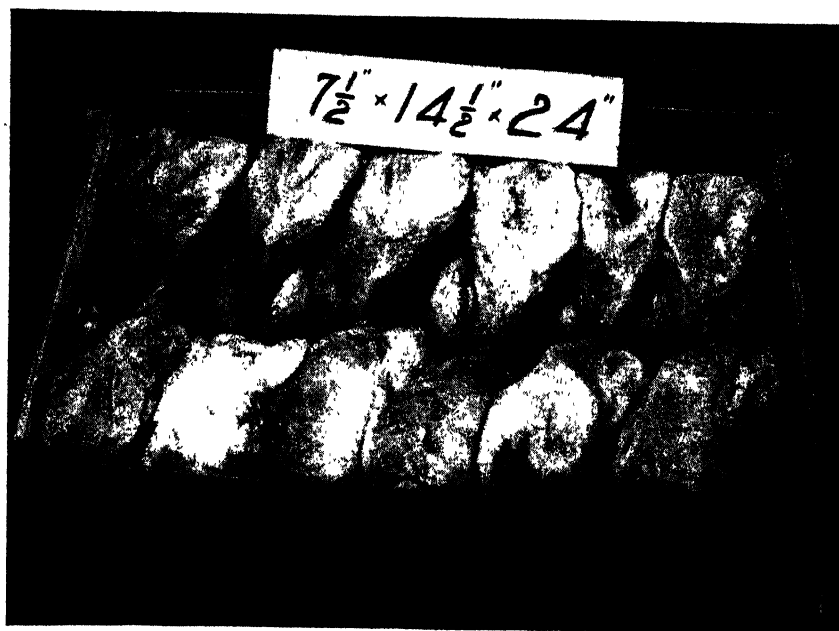
FIG. 1.   *Current method of packing poultry: weight of birds, 60 lb. per doz.*
FIG. 2.   *A suggested method of packing poultry: weight of birds, 60 lb. per doz.*

of another. Necks and legs in each row were alternated to reduce the space required. This pack fitted into a box with internal dimensions of 21 by 8 by 7½ in., i.e., approximately 1250 cu. in.

Both of these methods effect considerable saving in space and in packing material but have some disadvantages. In the common method of packing, the frozen birds can be separated fairly readily for sale, but in both the suggested methods the birds freeze into a solid block and cannot be separated readily unless completely defrosted, or unless two sheets of a light, dry-waxed paper are placed between the birds. The first of the suggested methods presents an unsightly appearance because of the protruding necks and joints; the second suffers from an objection to piling birds on top of each other. Neither method permits easy grading. However, these disadvantages are offset by the advantages; ease of evisceration, saving in packing material, and saving in space. In addition, the reduction of exposed surface should help reduce the freezer-burn that occurs during extended storage periods.

Freezing Poultry

Measuring Freezing

Several studies of factors affecting freezing rates in poultry have been made (5; 6; 7; 9, p. 438) and, in these, freezing time was defined as the time required for the temperature to be reduced by a definite amount. Development of a dynamometer for testing hardness and depth of freezing in frozen foods (8) permitted a comparison between these factors and freezing time as assessed by conventional temperature measurements.

In this phase of the study, three- and five-pound birds at about 31° F. were hung at intervals of 1 ft. in rooms operating at − 28°, − 10°, 0°, and 20° F., with gentle air motion. Thermocouples were placed in the centers of the birds and the freezing time was that required to attain a temperature of 20° F. in the center of the bird. If poultry flesh is similar to the flesh of other animal products (6) about 10% of the water would still be unfrozen at this temperature. Freezing times by this method were determined with a standard deviation of ± 0.6 hr. and were, therefore, considered satisfactorily reproducible.

Dynamometer measurements were made at each sampling time in three parts of the birds' carcass: in the breast area, between the lower ribs, and in the abdomen. There was little difficulty in allowing for passage of the dynamometer through the bones; but air cavities in the viscera were a problem, since they offered no resistance to the dynamometer. The best point for measurement appeared to be between the lower ribs, but the results presented here are the average for all three sets of readings.

The temperature measurements (Fig. 3) showed that increasing the temperature of the freezing chamber from − 28° to 0° F. increased the freezing time from 5 to 12 hr; at 20° F. the freezing time was about 48 hours.

The times in which the birds were frozen to any fixed depth (assessed by the dynamometer) paralleled the freezing time; and the hardness of the frozen portion, as measured by resistance to dynamometer penetration, increased uniformly as freezing progressed at any given temperature. The marked variation in hardness of birds frozen at different temperatures had no effect
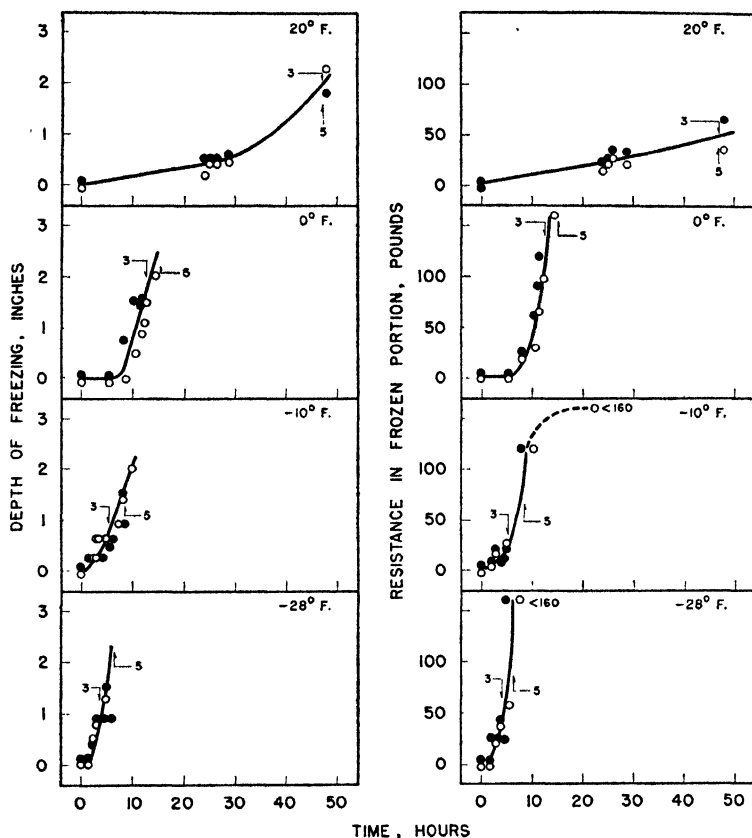

FIG. 3. *The freezing of 3-(o) and 5-(•) lb. chicken carcasses, as assessed by an impact dynamometer, and as related to the time for the center of the carcass to reach 20° F. determined with thermocouples: arrow marked 3, point at which 3-lb. birds reached this temperature; arrow marked 5, point at which 5-lb. birds reached this temperature.*

on the detection of unfrozen portions, which have no resistance to the dynamometer. It was concluded that, for poultry, the usefulness of the dynamometer will probably be confined to the detection of unfrozen spots in the carcasses.

Reducing Drip in Frozen Poultry

Studies elsewhere (7) have shown that open canals or vacuoles may be retained in muscle fibers by fast freezing. This retention of vacuoles was believed to be an indication of intrafibrillar freezing. Vacuoles may be present in the muscle fibers of live or freshly killed birds, although they may

not be detected by present histological techniques. Their presence in the muscles of rapidly frozen birds (7), and the reduced drip in defrosted birds that have been frozen rapidly (5), may be an indication that rapid freezing is necessary to preserve the vacuoles and, thereby, to reduce drip from the muscles after defrosting by retaining the fluid in vacuoles. If this is so, fluid might be retained by other procedures, such as the injection of solutions that would reduce pH changes, or alter the diffusion of ions that normally occurs in dead muscle tissue. This problem was examined by introducing various solutions into the birds' muscles, allowing the birds to stand for about an hour before freezing in about five hours, and by measuring drip after defrosting.

The solutions used were: water, 10^{-4} N ammonium hydroxide, phosphate buffer at pH 7.2 (3, p. 200), and Tyrode's modification of Ringer's solution (1, p. 257). The number of aliquots in any part of a carcass was roughly proportional to the amount of muscle believed to be in that portion of the bird and to a limiting amount of 30 ml. per lb. of muscle; i.e., its moisture content would be increased from approximately 70% to 85%. Each solution was used on groups of four birds; in addition four birds were frozen without injection, and four unfrozen birds were examined.

Drip was measured as the per cent decrease in weight by a technique similar to that described elsewhere (5) except that the right and left chest and thigh muscles were dissected from the frozen bird and drip measured without mincing.

The data were subjected to statistical analysis and the results are given in Table VI. Chest muscles lost a significantly greater quantity of fluid

TABLE VI

EFFECT OF ADDING 30 ML. OF VARIOUS SOLUTIONS PER LB OF BIRD, BEFORE FREEZING, ON THE AMOUNT OF DRIP IN DEFROSTED POULTRY MUSCLES

(Four birds given each treatment)

Treatment	Amount of drip, %		
	Chest	Thighs	Average
Unfrozen None	2 5	1 4	2 0
Frozen None	3 4	2 0	2 7
Water	5 4	4 0	4 7
Ammonium hydroxide	5 0	4 6	4 8
Phosphate buffer	4 6	4 2	4 4
Tyrode's solution	4 1	3 6	3 8
Necessary difference, 5% level			0 8
Average	4 2	3 3	

than thigh muscles, but, although frozen untreated muscle exuded more fluid than unfrozen muscle, the difference was not statistically significant. The injection of water into muscle caused a marked increase in drip, but a large portion of the added water was retained by the muscle. The use of Tyrode's solution gave a product with significantly less drip than that containing added water, indicating that retaining the proper ratio of salts and water in muscle may help to retain its structure during freezing. Evidence of vacuoles was found in frozen sections of only the muscles injected with Tyrode's solution.

Retaining Quality After Freezing

Frozen Storage

Poultry in chill or frozen storage develops an undesirable odor in the flesh of the thigh. It has often been suggested by processors and others that this odor was attributable to the short interval that elaspes between killing the bird and introducing it into the semi-scald tank. It was believed that the bird entered the water with its heart still beating, that the dirty semi-scald water containing bacteria was pumped through the veins and arteries, and that, during storage, bacteria retained in the thigh caused the off-odor. On the other hand, the possibility exists that off-odors come from the viscera, as only a thin membrane separates the viscera from the meat in the thigh.

To examine this question, Grade A fowl processed in an earlier phase of this study were stored at 0° and 30° F. and examined at the intervals shown in Fig. 4. Forty birds had been subjected to the 30-sec. bleeding time and 40 birds to the 90-sec. bleeding time. Each group of birds was divided into four lots: two lots from each group were eviscerated; one lot of eviscerated and one lot of noneviscerated birds from each group was stored at each temperature. At each sampling time random pairs of birds were drawn from each lot and group, and the bacterial count in the thigh muscles was determined. All the birds were submitted to a taste panel of 24 persons who smelled the eviscerated surface and the thigh meat and scored it according to a scale from 0, no off-odor, to 5, gross off-odor.

The viable bacterial count varied from 10^2 to 10^3 per gm. of thigh meat and did not change during storage at either temperature.

The taste panel data were subjected to statistical analysis and the significant results are given in Fig. 4. They showed that bleeding time had no effect on the development of off-odor either on the eviscerated surface or in the thigh meat, but that off-odors increased rapidly in the viscera and were transmitted to the thigh meat. Therefore, eviscerating seems to be a desirable step in processing frozen poultry.

Storing Defrosted Birds

The eviscerated surface of poultry is usually believed to deteriorate rapidly unless the carcass is frozen, and this deterioration is reputed to be more rapid in defrosted birds than in birds that have never been frozen. Therefore,
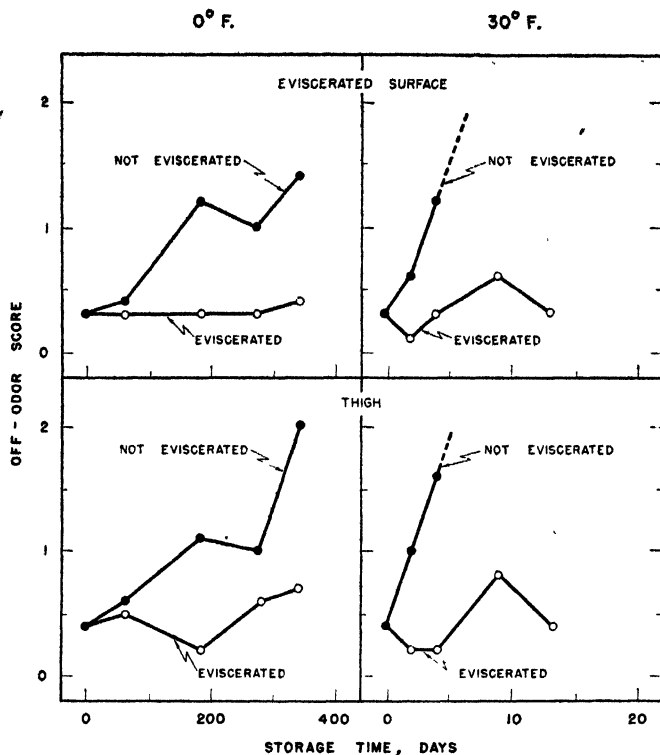
FIG. 4. *Change in off-odor on the eviscerated surface and in the thigh of eviscerated and non-eviscerated fowl stored at 0° and 30° F.*

it was of interest to determine the storage life of defrosted birds held at temperatures of 40°, 60°, and 80° F. and to examine possible methods of extending storage life at these temperatures.

For this study, birds weighing 60 lb. per dozen were eviscerated and treated before freezing as shown in Table VII. Wrapping was done by heat-sealing

TABLE VII

STORAGE LIFE, HR., OF DEFROSTED EVISCERATED POULTRY TREATED IN VARIOUS WAYS AND HELD AT DIFFERENT TEMPERATURES

Treatment	Temperatures, °F.		
	40	60	80
None	120	31	12
Partial cook	120	16	8
"Cellophane" wrap	48	25	12
Sodium benzoate dust	120	31	12
Butyl ester of *p*-hydroxybenzoic acid dust	120	31	12
Calcium proprionate dust	120	48	34
Carrageenin gel coat	168		
Carrageenin gel coat (4% sodium chloride)	168	72	
Carrageenin gel coat (6% sodium chloride)	192	83	32

the birds in 450 M.S.Y.T. "Cellophane". Carrageenin gel, an extract of Irish moss seaweed, was applied by dipping the birds into solutions, at 212° F., consisting of 4% of a 4 to 1 mixture of carrageenin and potassium chloride (4) to which 0, 4, and 6% of sodium chloride was added. The eviscerated birds were partially cooked by placing them in a chamber which was brought to 212° F. in 15 min. and held at that temperature for 30 min. The dry chemicals were dusted on the eviscerated surface only. Off-odors on the eviscerated surface were scored on a scale from 0 (good) to 4 (very badly off) and a score of 2 was considered the limit at which the birds would be suitable for table use. The numbers of viable bacteria on the eviscerated surface were determined at intervals, but the counts were too irregular to be of value.

• Table VII shows the time in hours required for the birds to reach an off-odor score of two. Unwrapped, untreated birds kept for 120, 31, and 12 hr. at 40°, 60°, and 80° F. respectively. Wrapping in Cellophane or partial cooking caused more rapid deterioration than holding untreated birds in the open. Dusting with dry chemicals had no effect. Coating the birds with carrageenin gel containing 6% sodium chloride approximately doubled the storage life. The gel is easily removed, and in this study, as in another study of the use of salt for preserving quality in unfrozen birds (10), the salt did not affect the palatability of the flesh.

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REFRIGERATOR CAR EXPERIMENTS. VII.

A ROAD TEST WITH A MECHANICAL CAR UNDER HEATING CONDITIONS

The mechanically refrigerated railway car built at the Pacific Fisheries Experimental Station at Vancouver in 1948 is described in an article in issue No. 77 of this series of Progress Reports, pp. 100-104. In that article it was intimated that the car is adaptable for heating as well as cooling so that it can be used for the transport of any perishable commodity requiring constant temperatures within the limits of the operation of the equipment; *i.e.,* between – 10° and +60°F. with the controlling mechanism presently being used.

On February 21st, 1949, the experimental car was loaded with eight hundred 50-lb. boxes of apples at Kelowna, B.C., consigned to Montreal, Que. With the thermostats (temperature-controlling mechanisms) set at about 33°F. and the switches set to "heating," the test commenced in mid-afternoon and ended approximately 7½ days (182 hours) later at Montreal on March 1st.

For the benefit of those not familiar with the means of heating by use of refrigeration equipment on the reverse cycle, or by what is popularly called the "Heat Pump" principle, and who may not have access to recent engineering handbooks for details of the operation, the following brief outline of the principles is given.

There are four essential elements in all compression systems of refrigeration, namely: the evaporator, the compressor, the condenser and the receiver. In the normal operation, the temperature of the evaporator is reduced *below* that of its surroundings by the evaporating refrigerant, therefore heat "flows" into it. This heat is taken up by the refrigerant which is thus changed from a liquid to a gas, and in its gaseous state the refrigerant is "pumped" by the compressor into the condenser. The condenser thereby becomes *hotter* than the air surrounding it, and gives up not only the heat taken in by the evaporator, but also that added at the compressor. In giving up this heat to the condenser, the gaseous refrigerant, under the increased pressure maintained by the compressor, is condensed into its liquid state and flows into the receiver where it is again ready to start its cycle at the evaporator.

Therefore if we want to *cool* any given space by this system we merely place the *evaporator* in that space; but if we want to *heat* that space we place the *condenser* in it. Since there is no essential difference between an evaporator and a condenser, there is no reason why, in a given system, they cannot be so designed that they may be used in either capacity. So that if we wish to *heat* the air in the space occupied by the evaporator, instead of changing the position of the evaporator, we merely convert it into a condenser by means of a system of cross-over refrigerant connections. This is the principle we have applied in our experimental car, and the suitability of the popular term "Heat Pump" is readily appreciated because we can "pump" heat *into* or *out of* the car merely by the manipulation of valves

in the refrigerant circuits. This manipulation can be manual or automatic. In the eventual operation of our car we hope to have all valves controlled automatically so that the change-over from "heating" to "cooling" will take place automatically as the desired conditions demand.

It may be argued that this automatic change-over is an unnecessary refinement, but during the road test just referred to we had occasion to change the operation from heating to cooling one afternoon, when the outside temperature rose to 43°F. and our thermometers indicated that the temperature in the car was rising above 36°F., the upper limit suggested by the Department of Agriculture.

Before summarizing the results of the recent road test, the number and locations of the thermometers will be explained. The previous article in this series explained that our car is completely "jacketed," therefore any temperature variations in the car will be induced mainly by changing conditions outside the jacket, since the apples supply a constant "flow" of heat at a given temperature equal to their heat of respiration at that temperature. Since we wished to assess the degree of success attending our efforts to distribute evenly the air circulation in the jacket, we placed the sensitive elements of our various temperature-indicating devices (thermocouples and resistance thermometers) at different points around the periphery of the load, paying particular attention to both ends of the car, where air circulation might be the most sluggish. In all we had twenty thermometers in the loading space of the car: six resistance thermometers placed top and bottom in three positions along a longitudinal vertical plane down the centre of the car, and fourteen thermocouples distributed evenly over both ends of the car. In addition, we had two resistance thermometers in the air stream in the jacket: one in the air entering the heating unit, and one in the exit air. Of course the car was equipped with the standard liquidometers, and since these were in the air stream in the jacket, they did not truly indicate the temperature conditions in the loading space. The temperature outside the car was obtained chiefly by means of a dial thermometer; but when the sun was shining, readings in the direct sun's rays were taken by a mercury thermometer in addition to the shade temperatures given by the dial instrument.

Since the scope of these Progress Reports does not cover detailed analyses of technical data, merely a summary of the significant results will be given here. Of main interest was the temperature at the coldest and warmest points in the car, where our thermometers were situated, as related to the outside temperature. The accompanying temperature graph is an attempt to present these data most conveniently in their proper proportions.

It will be noticed that the outside temperature varied between +43 and −10°F., giving an average of 16.2°F., while the coldest point in the car as indicated by our resistance thermometers (not necessarily the same thermometer throughout) varied between 31.4°F. and 34.8°F.; the warmest point varied between 34.1° and 36.5°F. The maximum difference between the cold and the warm points at any given time throughout the trip was about 4°F.

During the entire 182 hours of the test trip the mechanical units operated 36 times for various periods of from 2 to 15 minutes each, making up a total running time of 210 minutes. Thus their average running period

Curves A&B represent
temperatures on the longitudinal
centre plane of the car
Curve A- highest of 6 readings
Curve B- lowest of 6 readings

Outside Air Temp.

Time- Days → Feb. 21 to Mar.1

Midnight Noon MN N MN N MN N MN N MN N MN N MN
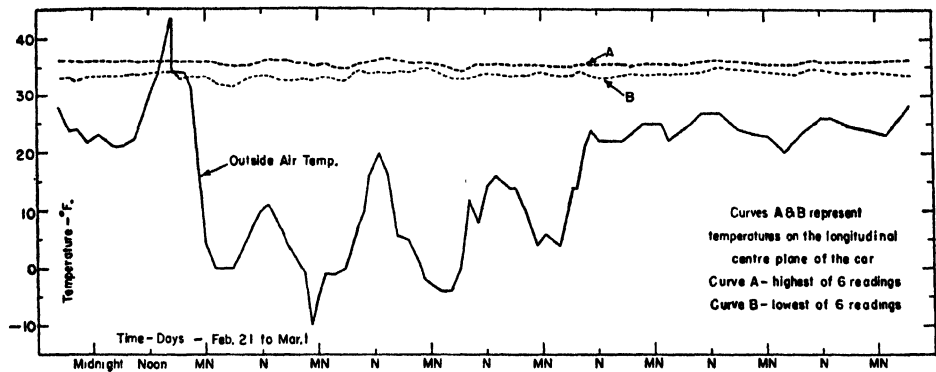
was about 6 minutes every 5 hours. Of this 210 minutes of running time, 9 minutes was for cooling the car when the external temperature went up to 43°F.

The gasoline consumption could not be conveniently measured, therefore the amount used can only be estimated. Operating periods of short duration, particularly when the starting is under cold conditions, do not give normal efficiencies; therefore, although the units may only require about 0.6 gallons each per hour under continuous operation, that rate might have been increased by at least 25% to say 0.75 gallons per hour each under the test conditions. On that assumption the amount of gasoline consumed was 5¼ Imperial gallons for the external weather conditions encountered during this trip.

Both practically and technically this test trip was highly successful. It demonstrated that the equipment will withstand ordinary freight train treatment, and that the temperatures desirable for fresh fruits and vegetables can be produced and maintained under freight treatment by the system employed. Although the weather conditions did not impose a severe test on the equipment, the performance under the conditions encountered was sufficiently convincing to lead us to believe that the desired car temperatures can be maintained by this system under any conditions that may be encountered in Canada. From the technical viewpoint the trip was also fruitful since the observations led to suggestions for modifications and refinements in design that may prove very beneficial in future experiments.

By way of illustration, several examples will be given: (1) The 4°F. difference in temperature between the coldest and warmest points in the car experienced on several occasions was, we believe, due to stratification of the air in the jacket resulting from prolonged periods of idleness of the machinery. Several very small fans operating from the batteries of the units would keep the air in the jacket agitated and thus keep the temperatures there more uniform. (2) Although the jacket is made up of a number of ducts, and we normally expect the temperature of the air in the ducts to change as the air picks up or gives up heat, our instruments indicated in this test that the mean temperature at the floor at the end of the car nearest the mechanical units was approximately 0.8°F. warmer than that at the other end, while the top mean temperature was about 0.6° warmer

than that remote from the units. In the light of the design of the car; reasoning will show that we have failed somewhat in inducing uniform air circulation throughout the jackets to give the desired results. Granted we would expect the mean temperature near the floor at the end remote from the units to be *colder* than that close to the unit, since the air will be giving up heat as it traverses the jacket, still the temperature near the top at that same end should be *warmer* than that at the close end for the same reason. In this road test we opened vents between the jacket and the interior of the car, to permit a proportion of the circulating air to pass through the load of apples to take up their heat of respiration. This could account for some of the anomalous results obtained, and will lead to further study.

In the next two months studies will be made of all the modifications necessary to bring the performance of the car up to a still higher level. Then, in the warm summer weather we expect to transport a carload of frozen fish east from the Pacific coast, and to bring a carload back from the east coast possibly to Vancouver. If our plans materialize, by the fall of 1949 the stationary and road tests should have produced sufficient data to reveal any weaknesses in our application of the principles involved, and should indicate the factors that may require any further special investigation to prove the practicability of the mechanical system of inducing controlled conditions in railway refrigerator cars.

Acknowledgments

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THE SEPARATION OF ISOLINOLEIC ACID FROM HYDROGENATED LINSEED OIL BY CHROMATOGRAPHIC METHODS[1]

By H. W. Lemon

Abstract

When linseed oil or other oils containing linolenic acid are hydrogenated, an isomeric linoleic acid (isolinoleic acid) is formed. Its concentration increases to a maximum, then decreases as hydrogenation proceeds. In view of the possible relationship of this acid to "flavor reversion", its separation in pure form has been investigated. Concentrates of isolinoleic acid or its methyl ester were obtained from partially hydrogenated linseed oil by crystallizing a large proportion of the more saturated acids or methyl esters from a solvent at low temperatures. Further fractionation of such concentrates by chromatographic methods was investigated. Silica gel was found to be better for the purpose than either activated alumina or activated carbon. Methyl isolinoleate was adsorbed more firmly on the silica gel than the less unsaturated esters, which were eluted by means of large volumes of hexane. Subsequent elution with chloroform removed the methyl isolinoleate.

Introduction

When linseed oil or other oils containing linolenic acid are hydrogenated, an isomeric linoleic acid is formed in which the double bonds are in such positions that diene conjugation is not produced by high-temperature saponification (1, 15). In a typical hydrogenation of linseed oil, the concentration of isolinoleic acid reached a maximum of 18% of the total fatty acids at an iodine value of approximately 100, then decreased on further hydrogenation.

Evidence has been presented that decomposition of isolinoleic acid may give rise to the characteristic odor and flavor that develops in partially hydrogenated linseed oil, particularly on heating (15, 16). This deterioration is commonly called "flavor reversion".

In view of the possible relationship of isolinoleic acid to flavor reversion, the separation of the acid in pure form by (a) fractional crystallization from a solvent at low temperature and (b) chromatographic methods has been investigated. The first method has been applied successfully by Brown *et al.* to the separation of a number of pure fatty acids (3, 4, 10, 11). The second method has been investigated by Cassidy (5, 6, 7), Kaufmann (13, 14), Graff and Skau (12), Dutton (9), Swift, Rose, and Jamieson (19), Riemenschneider, Herb, and Nichols (18), Claesson (8), and others. It was previously reported

(15) that an isolinoleic acid concentrate of iodine value 152 was separated by the crystallization procedure of Brown *et al.*, but attempts to separate the pure acid by this method were not successful. Therefore further purification of the concentrates by chromatographic methods has been tried, and this work will be described in this paper.

Experimental

Linseed oil hydrogenated to an iodine value between 75 and 80, and containing no trace of linolenic acid, as indicated by the analytical method of Mitchell, Kraybill, and Zscheile (17), was saponified and the mixed fatty acids were separated. A 5% solution of the mixed acids in acetone was cooled to $-20°$ C. while being agitated; this caused considerable crystallization. Filtration was achieved by suction, using a porous stone immersed in the mixture. The crystals were washed with a little acetone at $-20°$ C., the mixture filtered, and the filtrates combined. This solution was treated in the same manner, but at $-50°$ C., and the resulting filtrate was also treated in the same manner, but at $-65°$ C. The filtrate was now concentrated to about one-quarter of its volume and again allowed to crystallize at $-65°$ C. Acetone was removed from the final filtrate by distillation, and the resulting isolinoleic acid concentrate had an iodine value of 150 to 155. Attempts at further purification by crystallization were not successful. Some of the isolinoleic acid concentrate prepared in this way was converted to the methyl esters and distilled *in vacuo*.

A concentrate of methyl esters that proved to be a satisfactory starting material for chromatographic separations was obtained more simply, in the following manner. The hydrogenated linseed oil was converted to methyl esters by alcoholysis, using the method of Wright, *et al.* (20). A solution of 100 gm. of esters in 400 ml. of hexane was stored in a cabinet at $-18°$ C. for 18 to 24 hr. The mass of crystalline esters was separated by filtration, the filtrate concentrated, chilled to $-70°$ C., and again filtered. Esters with an iodine value of about 120 were obtained.

Activated alumina, activated carbon, and silica gel were used as adsorbents. The alumina samples used were chromatographic grades with an activity equivalent to 1 or 2 on the Brockmann scale (2). Samples of activity 4 and 5 on this scale were prepared from these by exposure to moist air.

The activated carbon used was Darco G 60, manufactured by Darco Corporation, New York. It was mixed with an equal weight of Johns-Manville Hyflo Super-cel.

The silica gel was a commercial grade, 28–200 mesh, obtained from the Davison Chemical Corporation, Baltimore, Maryland.

The adsorbents were packed in glass columns and were flooded with the solvent to be used. The solution of fatty acids or esters was then added to the top of the column. Large volumes of solvent were allowed to pass through the adsorbent, the eluate was collected in fractions, the solvent was

removed by distillation, the residue weighed, and refractive index and iodine number determinations made when possible.

Results

(a) *Activated Alumina*

A number of experiments were tried using activated alumina of various activities, and various ratios of methyl esters to alumina. The solvent was petroleum ether. Methyl isolinoleate adhered most firmly to the alumina, and was difficult to remove. It is probable that a method could be developed with the use of this adsorbent, but, owing to the difficulties encountered, it was abandoned.

(b) *Activated Carbon*

Fractionation of both fatty acid and methyl ester concentrates by means of activated carbon was investigated. In both cases, a fraction with a higher iodine value than that of the original concentrate came through the column at the beginning of elution with petroleum ether, followed later by more saturated fractions. The results of one experiment using a concentrate of methyl esters are given in Table I. The process was very slow, and pure isolinoleic acid was not obtained by one passage of the acids or esters through the column.

TABLE I

SEPARATION OF METHYL ESTERS CONTAINING METHYL ISOLINOLEATE, BY ADSORPTION ON ACTIVATED CARBON

Contents of column: 15 gm. Darco G. 60 } mixed
                      15 gm. Filter aid
                      1 gm. of methyl esters, iodine value 141

Solvent— Petroleum ether

Sample No.	Volume of eluate, cc.	Weight of esters, gm.	Iodine value of esters
1 to 2	125	0 02	
3 to 6	100	0 21	162
7 to 10	100	0 22	151
11 to 15	125	0 12	128
16 to 26	275	0.16	

NOTE:— *Esters recovered 0 73 gm.*

(c) *Silica gel*

Silica gel proved to be the most practical of the three adsorbents. When petroleum ether or hexane was used as solvent, methyl isolinoleate in a methyl ester concentrate was more firmly adsorbed than the more saturated esters, which were separated from the silica gel by elution with large volumes of the solvent. The adsorbed methyl isolinoleate was released by passing chloroform through the column, and the esters obtained had an iodine value close to the theoretical value of 172.3 for the pure ester. The ratio of esters to adsorbent was 1 to 10. The results of three experiments are given in Table II.

TABLE II

SEPARATION OF METHYL ISOLINOLEATE BY ADSORPTION ON SILICA GEL

Volume of eluate, cc.	Weight of esters eluted, gm.	Refractive index, 20° C.	Iodine value

Experiment I. 20 gm. silica gel. 2.1 gm. methyl esters (iodine value, 144). Solvent: petroleum ether, followed by chloroform.

Petroleum ether			
700	0.016	1.4566	
500	0.119	1.4530	
500	0.118	1.4539	
500	0.179	1.4545	
500	0.178	1.4549	
500	0.166	1.4560	
500	0.159	1.4570	
1000	0.162	1 4601	
1200	0.143	1.4608	
Total 5900	1.240		
Chloroform			
100	0.703	1.4615	173
100	0.038	1.4650	
100	0.049	1.4670	
Total 300	0.790		

Experiment II. 250 gm. silica gel. 25 gm. methyl esters (iodine value, 145). Solvent: petroleum ether, followed by chloroform

Petroleum ether			
65000	10.5	1.4546	103
Chloroform			
500	6.1	1.4606	167
500	3.4	1.4613	170
500	0.95	1.4616	174
500	0.46	1.4636	181

Experiment III. 250 gm. silica gel. 25 gm. methyl esters (iodine value, 117). Solvent: hexane, followed by chloroform

Hexane			
73000	16.1	1.4551	96
Chloroform			
500	3.2	1.4598	168
500	1.8	1.4606	170
500	1.0	1.4608	172
1500	0.8	1.4633	173
1000	0.3	1.4648	173

The steadily rising refractive indices of the fractions separated from the adsorbent in Expt. I by elution with petroleum ether show that the most saturated esters came through the column first, followed by fractions of increasing unsaturation, undoubtedly containing some quantity of methyl isolinoleate. It is important that elution with hexane or petroleum ether be continued no longer than is necessary to remove the more saturated esters if a good yield of methyl isolinoleate is to be obtained.

On eluting the column with chloroform, fractions varying in iodine number between 167 and 180 have been obtained. As the theoretical iodine number for linoleic acid is 172.3, it is obvious that there was a small amount of a more unsaturated substance present. The refractive index of the best methyl isolinoleate samples was approximately 1.4613 at 20° C. When elution with chloroform was continued, more viscous fractions with higher refractive index values were obtained. These may have been polymers.

The linolenic and linoleic acid content of the methyl isolinoleate concentrate, of the purified methyl isolinoleate from the adsorption column, and of the esters eluted from the column with hexane have been estimated by the spectral method (17). The amounts of these acids present were so small that accurate values were not obtained. In each case linolenic acid was of the order of 0.1% and linoleic acid about 1.0% of the total fatty acids.

The ultraviolet absorption of a solution of the purified methyl isolinoleate in isoöctane has been determined. This revealed that it contained a small amount of a substance having triene conjugation. The extinction value $(E \, _{1 \, cm}^{\%})$ at 268 mμ was 1.4.

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